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A Multifunctional Electrophoresis System

Specifications, Construction, and Operation

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C O N T E N T S

	Page
Acknowledgments	iii
Abstract	1
Introduction	1
Electrical and cooling network	2
Specifications and principle of operation	2
Power supplies	3
Constant-current regulator	3
Electrophoresis support station	14
Cart coolant system	28
Ancillary electrophoresis equipment	32
Vertical-slab-gel baths	37
Disk-gel baths	37
Disk-gel-casting stands and storage box	39
Disk-gel-column maker	39
Vertical-slab-gel cooling-chamber cells	42
Disposable vertical-slab-gel cells	42
Constant-temperature polymerization bath	50
Photopolymerization rack	50
Metal supports	50
Enzyme-reaction chambers	51
Rotary temperature bath	52
Gel measuring and drawing device	52
Costs	53
Procedures	54
Chemicals	54
Preparation of vertical-slab gels	55
Preparation of disk gels	55
Sample application	55
Electrophoresis	56
Postelectrophoresis steps	58
Performance	60
Discussion	60
References	61

I L L U S T R A T I O N S

Fig.		Page
1.	Schematic of the constant-current regulator, option 1	4
2.	Schematic of the electrophoresis support-station circuitry associated with the constant-current regulator	5
3.	Schematic of the constant-current regulator, option 2	6
4.	Schematic of the a.c. power-control safety-interlock system	7
5.	Components of the constant-current regulator, option 1	8

Fig.		Page
6.	Front panel of the constant-current regulator, option 2	9
7.	Constant-current regulator board	10–11
8.	Twelve-volt regulated power-supply board	13
9.	Current and voltage digital-voltmeter power-supply boards	15
10.	Construction details of the electrophoresis support-station frame ...	18
11.	Optional circuitry for the electrophoresis support station	19
12.	Construction details of the translucent face of the electrophoresis support station	20
13.	Construction details of the shelves used for vertical-slab-gel and disk-gel apparatus	21
14.	Working setup of the multifunctional electrophoresis system, option 1	22
15.	Working setup of the multifunctional electrophoresis system, option 2	23
16.	Construction details of the cart used for mounting the multifunctional electrophoresis system	25
17.	Details of the cart sliding doors that cover the front of the electrophoresis support station for operator safety	26
18.	Construction details of the coolant-valving station	27
19.	Construction details of the constant-flow manifold	29
20.	Construction details of the return manifold	30
21.	Construction details of the double-walled coolant-system box	31
22.	Schematic showing the flow pattern of the coolant system	33
23.	Construction details of the upper and lower vertical-slab-gel baths .	34
24.	Construction details of the upper and lower disk-gel baths	35
25.	Wiring diagram of the lower disk-gel baths	36
26.	Holding stand for 72 upper electrodes used with the disk-gel baths .	37
27.	Four apparatus that can be simultaneously run on the electrophoresis support station and controlled by the constant-current regulator ...	38
28.	Disk-gel-casting stand	39
29.	Construction details of the disk-gel-column maker	40
30.	Construction details of the vertical-slab-gel cooling-chamber cells ..	41
31.	Manufacture of the disposable vertical-slab-gel cells	43
32.	Gluing frame	45
33.	Disposable vertical-slab-gel cells and associated apparatus	46–47
34.	Cell-top positioning frame	48
35.	Constant-temperature polymerization bath	49
36.	Gel photopolymerization rack	50
37.	Metal supports	51
38.	Enzyme-reaction chamber	51
39.	Rotary temperature bath	52
40.	Gel measuring and drawing device	53
41.	Polyacrylamide-gel fractionation patterns of human serum control run simultaneously on 12 vertical-slab gels	57
42.	Polyacrylamide-gel fractionation patterns of human serum control run by the disk-gel technique with the separate upper and lower bath apparatus	58
43.	Polyacrylamide-gel fractionation of various protein standards	59

TABLES

	Page
1. Edge connector pinouts for constant-current regulator board	12
2. Edge connector pinouts for 12-V regulated power-supply board	14
3. Edge connector pinouts for current digital-voltmeter power-supply board	14
4. Edge connector pinouts for voltage digital-voltmeter power-supply board	14
5. Edge connector pinouts for current digital-voltmeter board	16
6. Edge connector pinouts for voltage digital-voltmeter board	16
7. Parts list for the constant-current regulator, electronic components .	16
8. Construction time and cost estimate for building the multifunctional electrophoresis system	54

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A Multifunctional Electrophoresis System

Specifications, Construction, and Operation

By Andrew C. Terranova¹

ABSTRACT

The multifunctional electrophoresis system consists of an integrated electrical network and ancillary electrophoresis equipment. The major components of the electrical network consist of commercially available high-voltage power supplies, a constant-current regulator, a refrigerated cooler or support cart, an electrophoresis support station, and an a.c. power-control safety-interlock system. These electronics allow up to 12 vertical-slab gels and 72 disk gels of various sizes to be electrophoresed individually and simultaneously at a constant current. Both cathodal and anodal runs are simultaneously possible with current and voltage being monitored at each of 12 stations. Either constant-voltage or constant-power electrophoresis is also possible with this multifunctional system. The ancillary equipment consists of various electrophoresis aids designed primarily to process efficiently and economically a large number of samples for studies with isozymes in insects. **KEYWORDS:** biochemistry, constant-current regulator, electrical engineering, electrophoresis, equipment (electrophoresis), gels (disk and vertical-slab), isoelectric focusing, isozymes, multifunctional electrophoresis system, physical chemistry.

INTRODUCTION

Electrophoresis and isoelectric focusing are becoming indispensable techniques for solving certain problems concerning the boll weevil, *Anthonomus grandis* Boheman, and the bollworm, *Heliothis zea* (Boddie), at the Cotton Insects Research Laboratory at Florence, S.C. These problems include the biochemical characterization of specific proteins, the genetic variability among and within insect populations in both

space and time, qualitative and quantitative protein differences in insects reared under different dietary and climatic regimens, the genetics of various polymorphic enzyme systems, and the eventual correlation of these parameters with the physiology and behavior of these insects.

Such broad research utilizing electrophoresis and isoelectric focusing requires that various forms of these techniques be used: disk gel, slab gel, micro gel, and preparative gel. Sample size ranges from a single, one-time-only determination to a multispecimen, mass-production scale. Different electrophoretic media, buffer systems, pH, running times, currents, and polarities are used, depending on the problem.

Although commercial equipment necessary to

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carry out these techniques is available, none of the apparatus have the ability to make simultaneous use of a combination of these methods. This report describes a system that allows full, simultaneous use of most forms of commercially available equipment and the many innovations suggested in the literature, and that also lends itself to the addition of new equipment and techniques. This equipment was designed so that it may be expanded in a modular fashion.

Although this report deals primarily with equipment developed for the use of polyacrylamide gels as the electrophoretic media, most of the equipment necessary for other electrophoretic media such as starch, agarose, and cellulose acetate strips will work equally well.

Most of the equipment has been used at two laboratories over the last 4 years. This report gives specifications, construction details, and operating procedures for this system as it now exists. The equipment was designed so that studies in the area of population genetics could be carried out effectively and economically.

Essentially, the system consists of an integrated electrical network and ancillary electrophoresis equipment. The major components of the electrical network consist of high-voltage power supplies, a constant-current regulator, a refrigerated cooler or support cart, an electrophoresis support station, and an a.c. power-control safety-interlock system. Each of these components was designed and built from commercially available resources, from ideas in the literature, and from onsite innovations.

ELECTRICAL AND COOLING NETWORK

SPECIFICATIONS AND PRINCIPLE OF OPERATION

The electronics of the multifunctional electrophoresis system was designed to meet the following specifications:

(1) Power 12 vertical-slab gels of various sizes individually at a maximum of 900 V at 35 mA each.

(2) Power 72 disk gels of various sizes individually at a maximum of 150 V at 3.5 mA each.

(3) Simultaneously electrophorese vertical-slab gels and disk gels in any combination.

(4) Operate in the constant-current mode with a minimum of drift.

(5) Operate in the constant-current mode with limited voltage for isoelectric focusing.

(6) Individually turn any of the 12 vertical-slab gels or 72 disk gels on or off without affecting the current to the other gels.

(7) Operate at 0 to 3.5 mA or 0 to 35 mA simultaneously with independent current adjustment for each of 12 stations.

(8) Independently reverse polarity at each station so that anodal and cathodal runs can be performed simultaneously.

(9) Monitor the current through each station.

(10) Monitor the voltage through each station.

(11) Use power supplies directly.

(12) Substitute many of the commercially available electrophoresis and isoelectric focusing systems into the complex.

To accomplish these specifications, additional circuitry had to be interposed between the commercially available high-voltage power supplies and the electrophoresis media. These electronics, mainly associated with the constant-current regulator (CCR), consist of three groups of four identical circuits. Each group is normally connected to one power supply. In this manner, each power supply can be used to operate from 1 to 12 vertical-slab gels, each with its separate upper and lower bath at up to 35 mA per gel, or from 1 to 24 disk gels, each with an upper and lower bath at up to 3.5 mA per gel in groups of 6 disk gels per circuit. The four groups of disk gels are connected in parallel, whereas each group of six vertical-slab gels is connected in series across the power supply. One vertical-slab-gel bath connected in parallel to the power supply can be substituted for each group of disk gels.

With this arrangement and at a constant current of 3.5 mA, each disk gel has a maximum capacity of a 150-V drop, or a 900-V drop per group of six, which is within the voltage capabilities of the high-voltage power supply and the CCR. Likewise, at a constant current of 35 mA, each vertical-slab gel has a maximum capacity of a 900-V drop. For isoelectric focusing, the high-voltage power supply can be set to limit the amount of voltage supplied for a given current to protect the gel from power levels that could cause excessive heating and result in sample destruction.

The high-voltage power supplies (HVPS) consist of three unmodified ISCO model 493 power packs capable of constant current, voltage, and power operation. The choice of these power supplies was made primarily with versatility in mind. Before this model became commercially available, other power supplies delivering at least 1,000 V constant voltage and 150 mA current were used for the majority of applications, although power supplies of lesser capability can and have been substituted for most applications with comparable results. A single power supply with a 5,000-V, 500-mA capacity (such as that manufactured by Savant Instrument Co.) can be substituted for the three separate power supplies, but with a loss of versatility.

If the full capabilities of individually powering 72 disk gels with upper and lower baths are not required, then numerous power supplies with constant-voltage capabilities at a minimum of 400 V and 360 mA can be substituted by connecting the three groups of CCR circuits in parallel with the power supply.

It is important to switch the power supply to the constant-voltage mode when the power supply is used in conjunction with the CCR. If this is not done, damage to the CCR or to the power supply is possible. Also, a high-voltage setting of 900 V is the practical upper level of the power supply because of the limiting factors in the CCR circuitry.

CONSTANT-CURRENT REGULATOR

Description

The versatility of the multifunctional electrophoresis system and the majority of the specifications listed are primarily derived from the constant-current regulator (CCR). A schematic of the overall CCR circuitry is presented in figure 1. A transistor (Q1) (fig. 1, sec. B), the limiting factor, is inserted in each of the four parallel paths on a power supply to act as a variable resistor for current control. The collector-to-emitter current of Q1 is the same current that passes through the electrophoresis cells and is determined by the voltage setting at the base of Q1. Because the current is determined by the base voltage, it will remain independent of the number

of cells turned on or of any variation in cell resistance. The stability of the current depends on the stability of the base current in Q1. To increase this stability, a second transistor (Q2) was added as an emitter follower to provide the current gain required to drive the current regulator Q1.

A low-voltage power supply (LVPS) (fig. 1, sec. A) is necessary to power the base circuitry of the constant-current regulator. This power supply uses transistor Q3 as a 12-V series regulator. The base of Q3 is held at 12 V by zener diode D3, and D4 is used for temperature compensation. The output of this supply is applied across potentiometer R8 to select the base current for Q2, which governs the range of current through which the regulator can be adjusted.

The electrophoresis cells (fig. 2) act as the collector resistor of Q1. When the disk-gel configuration is used, each of the six cells in the collector of Q1 has an on-off switch (S11-16) that, when in the off position, bypasses the cell (the voltage is not removed). As each cell is turned off, the collector resistance decreases. As this resistance decreases, the effective collector-to-emitter resistance of Q1 increases, and the voltage that was applied across a cell is now added to the voltage dropped by Q1. Also, during an electrophoretic run, the resistance through a cell changes. This change in resistance causes a corresponding change in the voltage dropped by the cell and therefore causes a current change through the cell. Transistor Q1 opposes these changes and thereby limits the current in the cell. Resistors R1 and R2 (fig. 1, sec. B), controlled by switch S5, serve as a coarse range selector, limiting the regulator range to either 0 to 3.5 mA or 0 to 35 mA.

When a vertical-slab gel is used in place of the six disk gels, it is convenient to attach it to the terminals (fig. 2) for the normally negative side of disk gel 1 and 6 (J11 and J16, respectively), turn switches S11-15 off, switch S16 on, and reverse polarity for an anodal run. A double-pole double-throw center-off switch (S6) is used for the on-off polarity-reversing switch, the polarity being reversed by an X-connection across the switch (fig. 1).

Two options have been used to measure the current through each station. Each requires a different circuitry. Option 1 (fig. 1) utilizes two digital voltmeters (DVM): one to measure the

(Continued on page 9.)

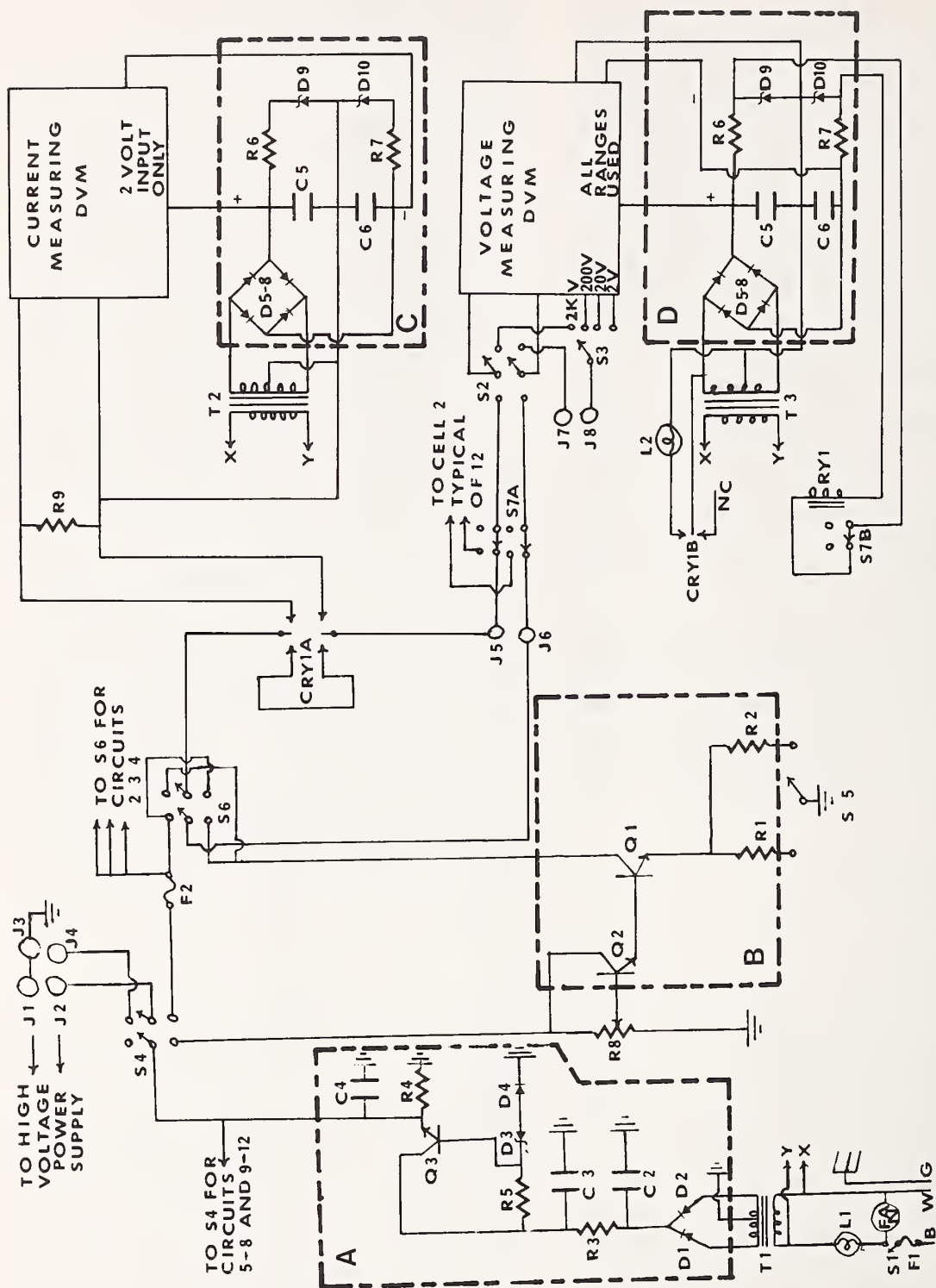


FIGURE 1.—Schematic of the constant-current regulator, option 1. A, 12-V regulated power-supply card (see also figure 8 and table 2); B, main regulator card, one of four circuits shown (see also figure 7 and table 1); C, current digital-voltmeter power-supply card (see also figure 9 and table 3); D, voltage

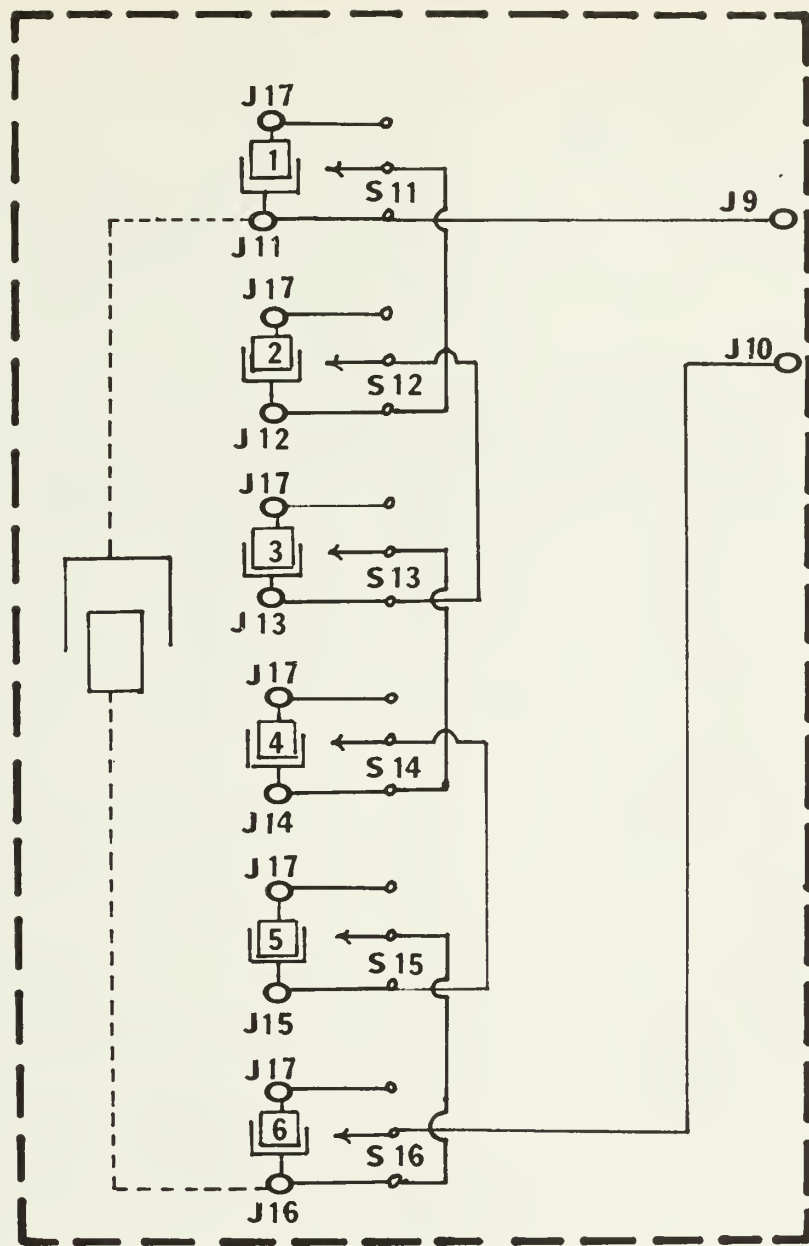


FIGURE 2.—Schematic of the electrophoresis support-station circuitry associated with the constant-current regulator.
 Note: Diagram shows only 1 of 12 identical circuits.

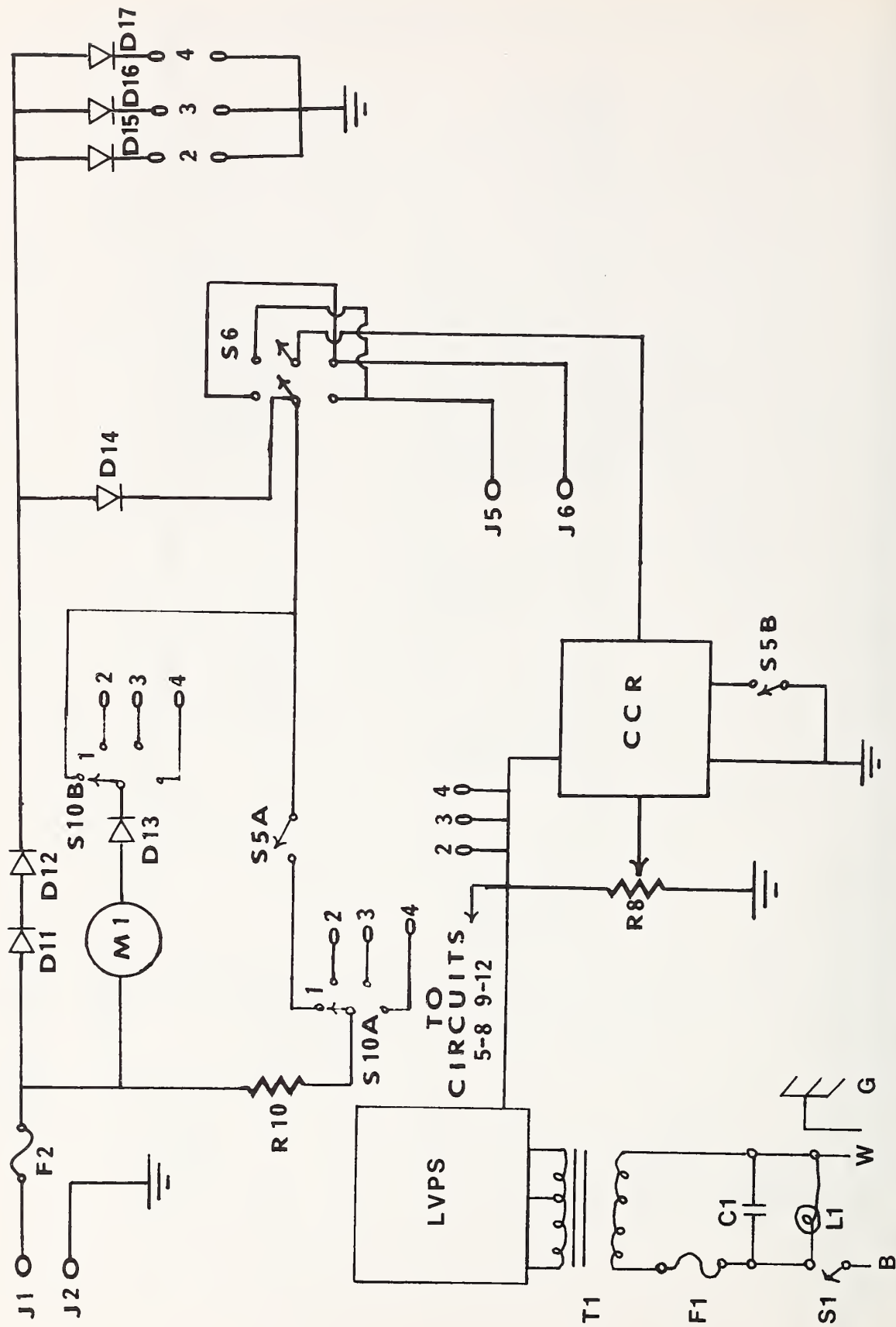


FIGURE 3.—Schematic of the constant-current regulator, option 2. Note: CCR=main regulator card, schematic of which is identical to figure 1B; LVPS=12-V regulated power-supply card, schematic of which is identical to figure 1A.

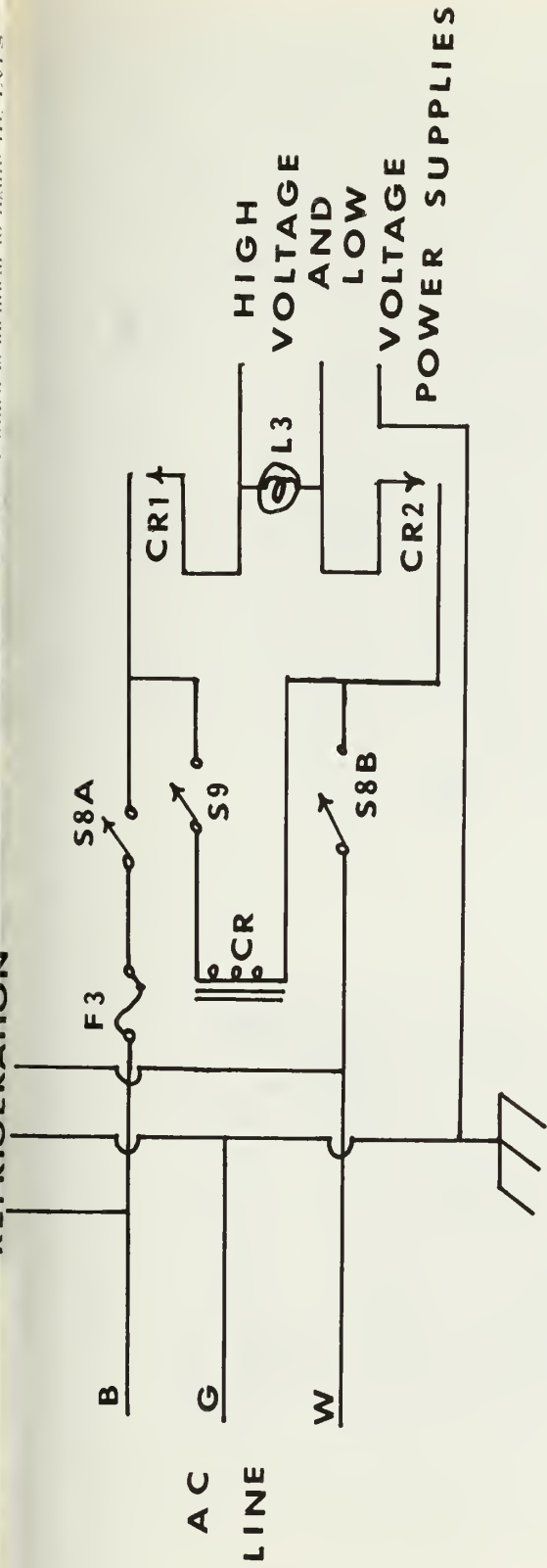


FIGURE 4.—Schematic of the a.c. power-control safety-interlock system.

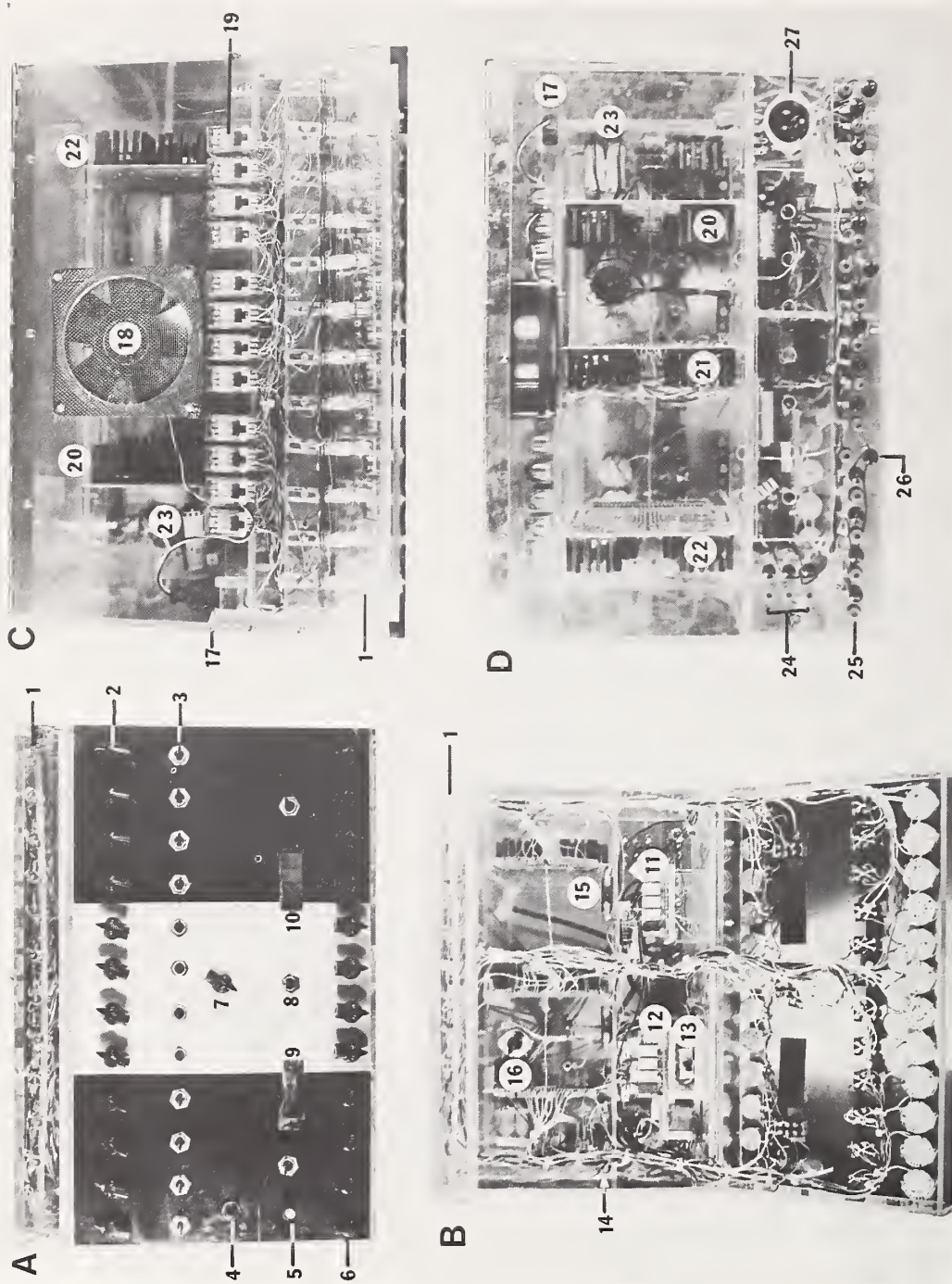


FIGURE 5.—Components of the constant-current regulator, option 1. A, Front panel; B, front with panel open; C, top view; D, back view. (1) Lamps (12) indicate meter select switch position; (2) X1 and X10 select switch (12); (3) normal-off-reverse switch (12); (4) low-voltage power-supply on-off switch; (5) pilot lamp; (6) current adjust control (12); (7) meter select switch (12 positions); (8) normal-bypass switch (3); (9) digital voltmeter; (10) digital current meter; (11) edge connector for digital current meter; (12) edge connector for digital voltmeter; (13) normal-test switch for digital voltmeter; (14) main power fuse; (15) fuse for individual regulator boards (3); (16) voltage select switch; (17) a.c. power source for fan; (18) fan; (19) relays (12); (20–22) regulator boards; (23) 12-V power-supply board; (24) bypass jacks (3 pairs); (25) output to individual cells (12 pairs); (26) input from high-voltage power supply (3 pairs); (27) a.c. power receptacle for line cord.

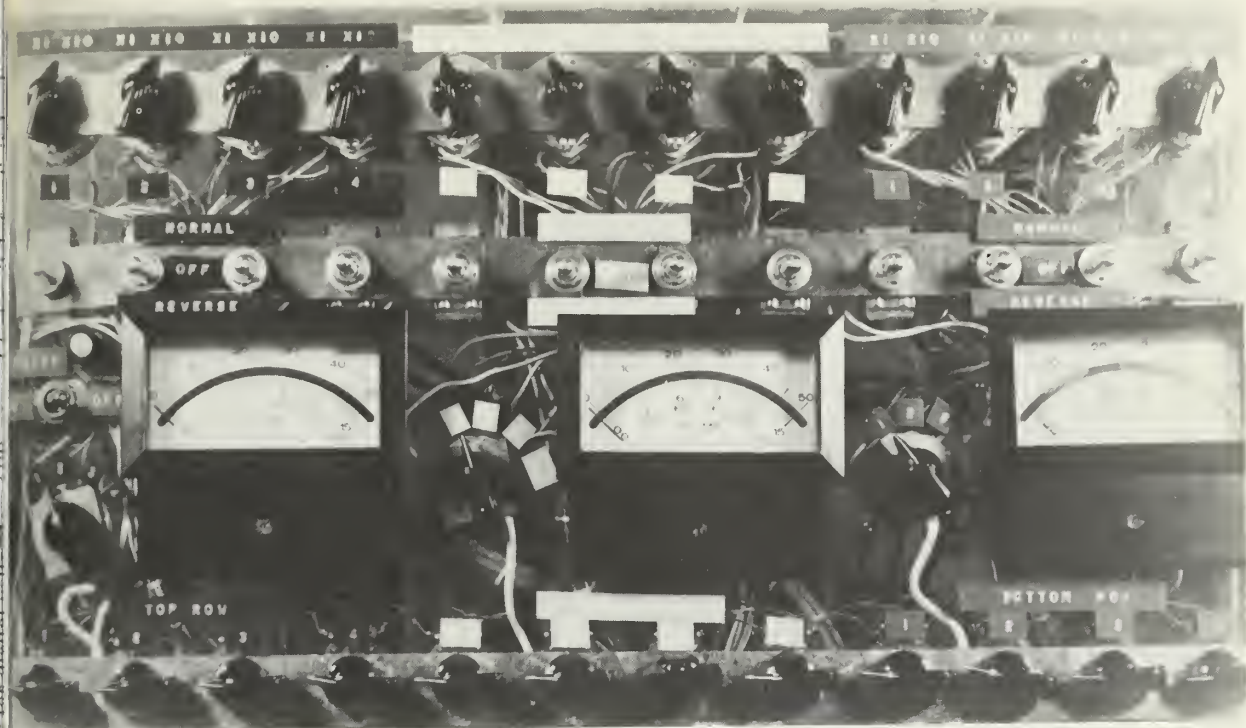


FIGURE 6.—Front panel of the constant-current regulator, option 2.

current passing through a cell, the other to measure the voltage of that cell (schematics of the digital voltmeters are not shown because they are included with the DVM kits used). Two power supplies are required for this option. The power supply used for the voltage-measuring DVM (fig. 1, sec. D) utilizes two 12-V zener diodes (D9, D10) in series to supply the 24 V necessary for the operation of relays (CrY). Resistors R6 and R7 are current limiting in function. The input from the transformer (T3) also supplies current for the bank of lights (L2) that indicate the circuit being monitored.

An optional circuitry associated with this DVM allows it to be used as a built-in voltmeter with ranges from 2 to 2,000 V selected by switch S3. Switch S2 isolates this circuit from the CCR circuitry. Two jacks, J7 and J8, are used to attach the test probes.

In the current-measuring DVM power supply (fig. 1, sec. C), the resistors R6 and R7 act as bleeders, and diodes D9 and D10 are not functional. Resistor R9 acts as a current-to-voltage converter.

Switch S7 is a 12-position switch used to select any one of the 12 circuits of the constant-current regulator to be monitored. The high-voltage power supplies are electrically connected to the constant-current regulator by means of jacks J1 and J2. A bypass circuit, controlled by switch S4, is connected to these jacks. In the normal position, the CCR circuitry is connected, and jacks J5 and J6 are provided for the cell leads; in the bypass position, the CCR circuitry is not functional, the signal from the high-voltage power supply going directly to jacks J3 and J4, allowing the HVPS to be used directly with any ancillary equipment without connection to the regulator or monitoring circuits.

The circuitry for option 2 utilizes three analog scales (fig. 3). In series with each transistor (Q1 of the CCR) is a diode (D14). Normally, during an electrophoretic run, there is enough voltage across D14 for it to conduct. However, to monitor the current through an electrophoresis station, a milliamperemeter (M1) of 3 ohms internal resistance is switched in parallel with D14 by

(Continued on page 12.)

CAUTION HIGH VOLTAGE CIRCUITS

FMP5
5172

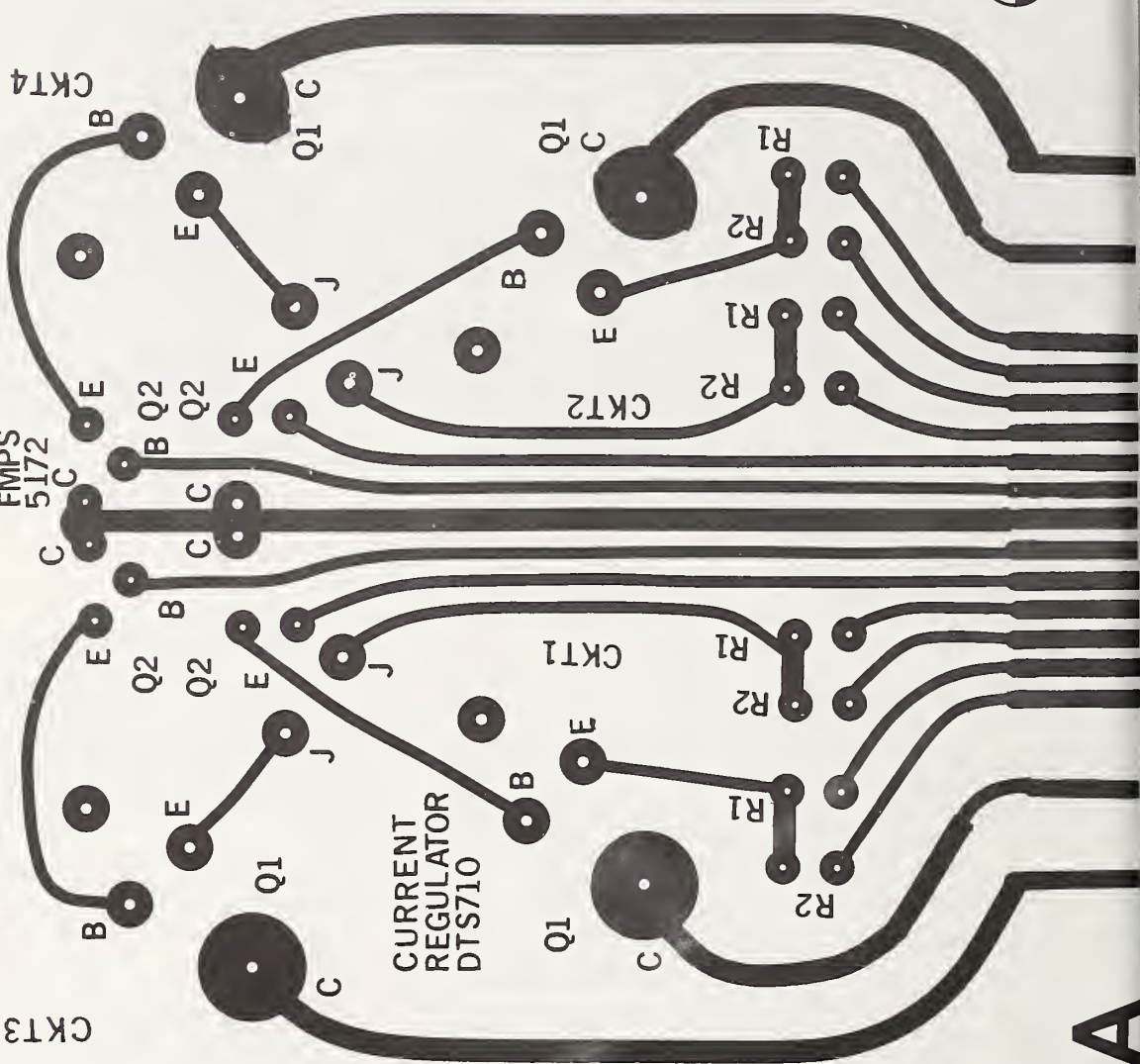
CKT3

CKT4

CURRENT
REGULATOR
DTS710

CKT2

CKT1



B

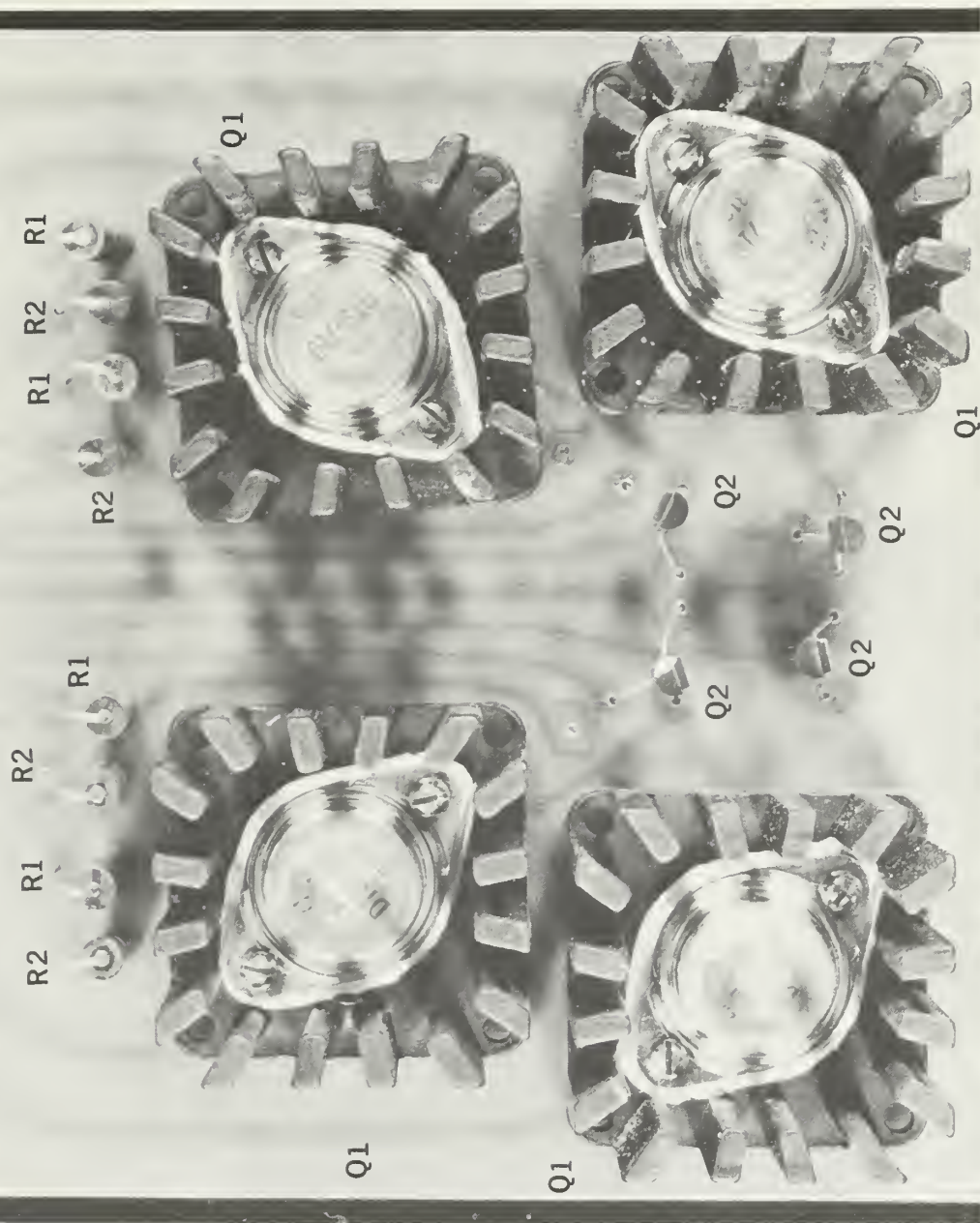


FIGURE 7.—Constant-current regulator board. A, Actual size etching and drilling guide (see table 1 for edge connector pinouts); B, mounting locations for components.

Table 1.—Edge connector pinouts for constant-current regulator board (fig. 7A)

Pin ¹	Function on board	Function external to board
1	Collector Q1, circuit 4	High voltage to S6, circuit 4.
4	Collector Q1, circuit 2	High voltage to S6, circuit 2.
7	R1, circuit 4	Ground of S5.
8	R2, circuit 4	S5, circuit 4.
9	R1, circuit 2	Ground of S5.
10	R2, circuit 2	S5, circuit 2.
11	Base Q2, circuit 2	Slider R8, circuit 2.
12	Base Q2, circuit 4	Slider R8, circuit 4.
13	Collectors of Q2, all circuits	+12 V, LVPS.
14	Base Q2, circuit 3	Slider R8, circuit 3.
15	Base Q2, circuit 1	Slider R8, circuit 1.
16	R1, circuit 3	Ground of S5.
17	R2, circuit 3	S5, circuit 3.
18	R1, circuit 1	Ground of S5.
19	R2, circuit 1	S5, circuit 1.
22	Collector Q1, circuit 1	High voltage to S6, circuit 1.
25	Collector Q1, circuit 3	High voltage to S6, circuit 3.

¹ No connection at pins 2, 3, 5, 6, 20, 21, 23, 24.

switch S10. At 200-mA maximum (the limit of the HVPS used), the 3 ohms of M1 does not drop enough voltage for D14 to conduct. Thus, when D14 is in parallel with M1, all the current in that branch passes through M1, giving an accurate reading of the current through the cell being monitored. A diode (D13) is connected in series with M1 to stop any reverse current through M1 when the low-voltage power supply is on and the high-voltage power supply is off. To compensate for the voltage dropped across D13, two more diodes (D11, D12) are connected in series with all the branches. For the higher range of current (0 to 35 mA), a shunt resistor (R10) is switched in parallel with M1. In both options, fuses F1 and F2 are provided to protect the circuitry of the constant-current regulator from damage in case of malfunction.

The a.c. power-control safety-interlock system (fig. 4), provided for operator safety, consists of a main fuse (F3), a master power switch (S8) that cuts both sides of the line, and a relay (Cr) that, in conjunction with a magnetic reed switch (S9), provides a safety shutdown of all high-voltage circuits. The switch is closed as long as it is in a magnetic field. If it is moved away from the center of the magnet, the reed-relay contacts open, and the current through the relay coil is interrupted, causing the contacts (Cr1 and Cr2) of the relay to open, removing all a.c. power ex-

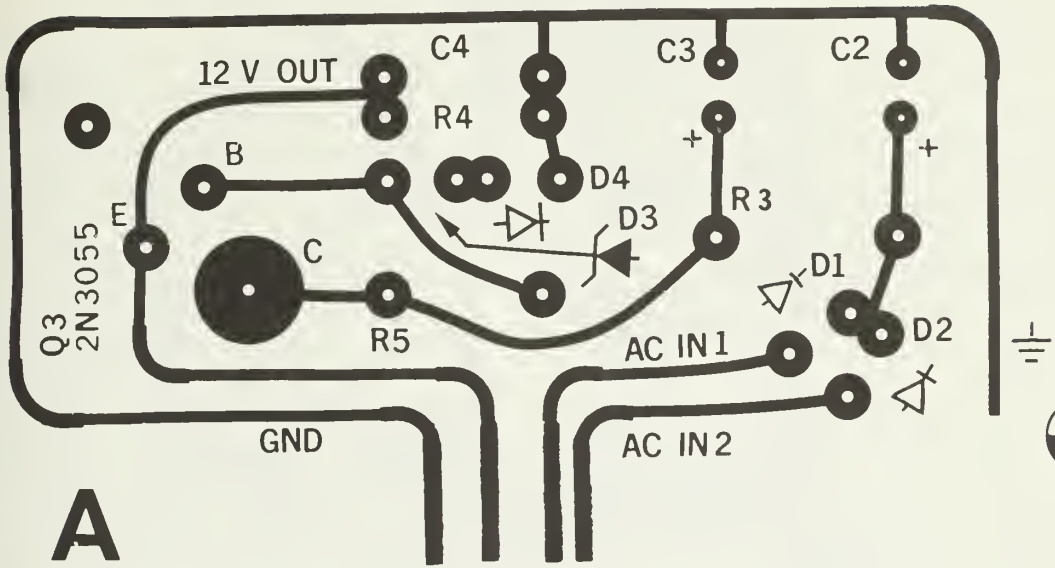
cept for that to the refrigeration system and to the fluorescent lights of the support station.

Construction details

Housing and controls.—The housing for the electrical components of the constant-current regulator is made of acrylic plastic. Figure 5, A–D, shows the construction details and the arrangement of the components in option 1, and figure 6 shows the front panel arrangement of option 2. Acrylic plastic can be quickly fabricated to meet size, shape, and electrical-insulating requirements. All regulator controls and the monitor meters are mounted on hinged front panels of the case. To make operation easier, these controls are grouped in horizontal rows by function and vertical columns by circuit association. A strap made from 1- by $\frac{1}{16}$ -in copper strip is used to ground each row of controls.

Constant-current regulator boards (high-voltage circuit boards).—Three identical plug-in circuit boards measuring 6 by 6 in are made from standard light-sensitive glass epoxy stock (fig. 7, actual size). Mounting holes for the regulator components are made by drilling (fig. 7A). The mounting locations for these components can be determined from figure 7B. The circuit boards are symmetrical about the center run (pin 13 of the edge connector), and components for four

12 VOLT REGULATED POWER SUPPLY



A

6 4 2 1

B

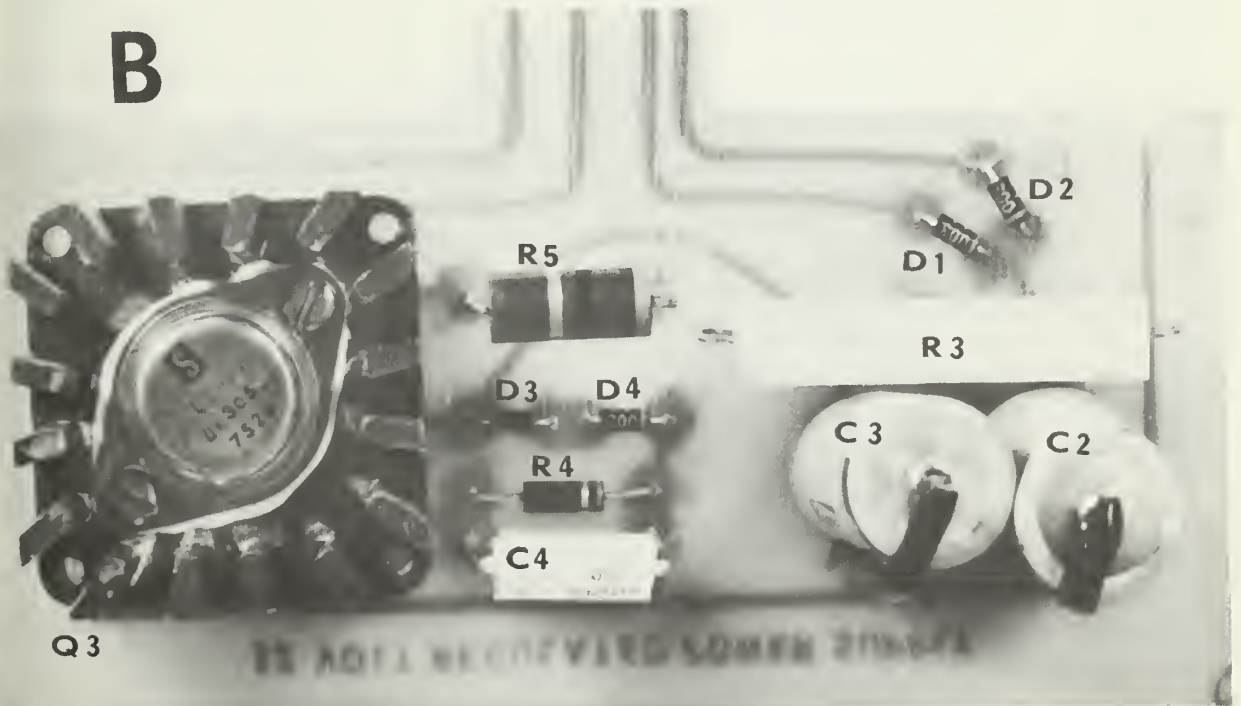


FIGURE 8.—Twelve-volt regulated power-supply board. A, Actual size etching and drilling guide (see table 2 for edge connector pinouts); B, mounting locations for components.

Description

The electrical leads (J1, J2) from the constant-current regulator pass to a wood and Plexiglas framework (fig. 10) designed to (1) illuminate by diffuse back lighting each of 12 electrophoresis stations, (2) support a variety of electrophoresis equipment by means of removable shelves, (3) hold the lead terminals and on-off switches for each of the 72 disk gel and 12 vertical-slab-gel stations controlled by the constant-current regulator, (4) hold the lead terminals and on-off switches for 2 other circuits distinct from the CCR circuitry, and (5) provide for an externally located cooling unit (used with specialized vertical-slab gels discussed later) and its inherent plumbing system which can be used to cool individually and independently each of the 12 vertical-slab-gel stations, when the electrophoresis system is not used in conjunction with the refrigerated cooler.

(Continued on page 19.)

Table 3.—Edge connector pinouts for current digital-voltmeter power-supply board (fig. 9A)

Pin ¹	Function on board	Function external to board
1	a.c. in	Transformer lead.
2	a.c. in	Transformer lead.
6	Ground	Ground and transformer circuit.
+	12 V out	12 V to DVM.
—	12 V out	12 V to DVM.

¹ No connection at pins 3–5.

Table 4.—Edge connector pinouts for voltage digital-voltmeter power-supply board (fig. 9A)

Pin ¹	Function on board	Function external to board
1	a.c. in	Transformer lead.
2	a.c. in	Transformer lead.
4	12 V out	12 V to relay (CrY).
5	12 V out	12 V to relay (Ry).
6	Ground	Ground and transformer circuit.
+	12 V out	12 V to DVM.
—	12 V out	12 V to DVM.

¹ No connection at pin 3.

circuits are located on each board (the circuits are numbered 1 through 4). In circuits 3 and 4, a wire jumper is connected between the two points designated "J." This jumper allows two runs (one from the junction of R1 and R2 to the emitter of Q1 in circuits 3 and 4, and the other from the emitter of Q2 to the base of Q1 in circuits 1 and 2) to cross over each other without electrical connection. Note that resistors R1 and R2 are mounted perpendicular to the board to give improved heat dissipation (fig. 7B). The heat sinks associated with Q1 are at collector potential, up to 900 V, and should not be touched when the unit is in operation. The edge connector pinouts for these circuit boards are given in table 1.

Twelve-volt regulated power-supply board.—The plug-in circuit board for the 12-V power supply is cut to 3 by 6 in from stock, and the components are mounted upon the foil pattern seen in figure 8A (actual size). The mounting locations can be determined by comparing the pad designations with figure 8B. Be sure to observe diode and capacitor polarity when installing these devices. Edge connector pinouts are given in table 2.

Current and voltage DVM power-supply boards.—The plug-in circuit boards for these power supplies are cut to 4 by 4 in and constructed in the same manner as described for the above boards. The component mounting locations are given in figure 9, and the edge connector pinouts in tables 3 and 4.

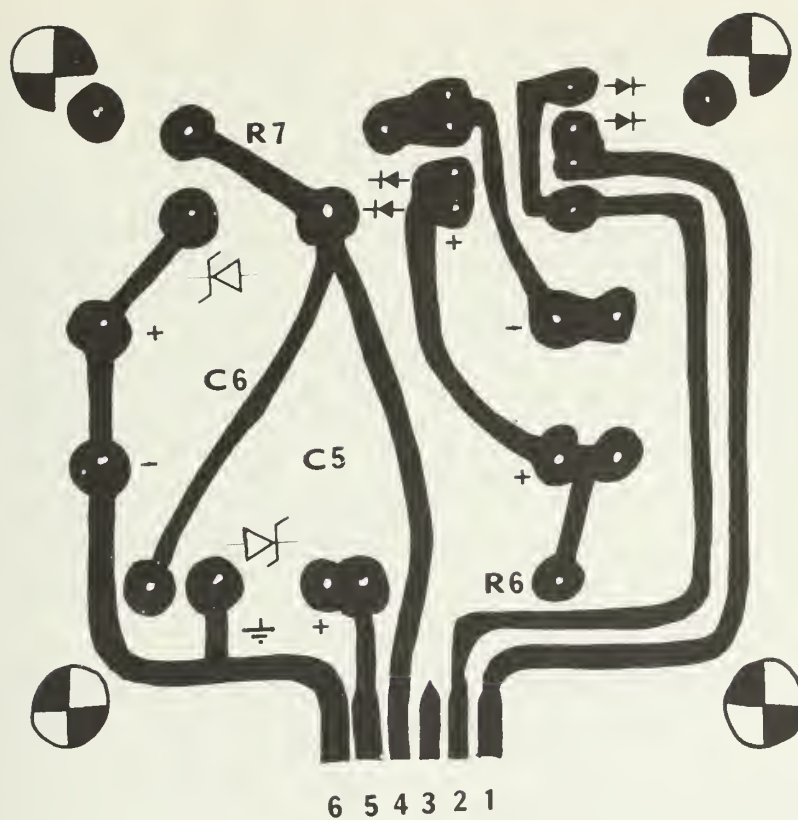
The 10-ohm resistor (R9, fig. 1) connected between pins 2 and 11 is used as a current-to-voltage converter by means of the 2-V input of the current-measuring DVM (see tables 5 and 6 for current and voltage DVM connector pinouts). A parts list for the constant-current regulator is given in table 7.

Table 2.—Edge connector pinouts for 12-V regulated power-supply board (fig. 8A)

Pin ¹	Function on board	Function external to board
1	a.c. in	Transformer lead.
2	a.c. in	Transformer lead.
4	12 V out	Regulated 12 V to F
6	Ground	Ground and transfo . . . circuit.

¹ No connection at pins 3, 5.

A



B

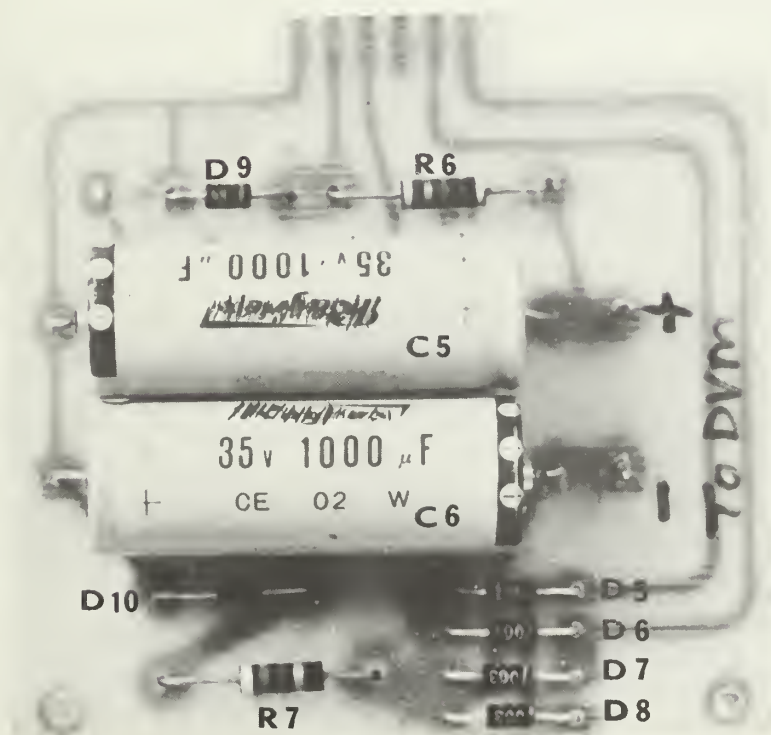


FIGURE 9.—Current and voltage digital-voltmeter power-supply boards. A, Actual size etching and drilling guide (see tables 3 and 4 for edge connector pinouts); B, mounting locations for components.

Table 5.—Edge connector pinouts for current digital-voltmeter board (see fig. 1)

Pin ¹	Function on board	Function external to board
1	12 V in	Positive post of current power supply.
2	Ground	Ground of Ry, R9, DP3.
3	12 V in	Negative post of current power supply.
7	Divider out	To 2-V circuit on DVM.
11	2 V	To Ry, R9, divider out.
13	DP3	Ground.

¹ No connection at pins 4-6, 8-10, 12, 14, 15.

Table 6.—Edge connector pinouts for voltage digital-voltmeter board (see fig. 1)

Pin ¹	Function on board	Function external to board
1	12 V in	To positive post voltage power supply.
2	Ground	Ground of S2.
3	12 V in	To negative post voltage power supply.
7	Divider out	To S3.
8	2 kV	To S2.
9	200 V	To S3.
10	20 V	To S3.
11	2 V	To S3.
12	DP4	To S3.
13	DP3	To S3.
14	DP2	To S3.

¹ No connection at pins 4-6, 15.

Table 7.—Parts list for the constant-current regulator, electronic components

Circuit designation	Description	Quantity	
		Option 1	Option 2
S1	Single-pole single-throw toggle switch	1	1
S2	2-Position, on-on, paddle-handle toggle switch	1	0
S3	2-Circuit, 1-section, 2- to 6-position, nonshorting rotary switch	1	0
S4	2-Position, on-on, paddle-handle toggle switch	3	0
S5	2-Pole, 2-position, 2-section ceramic switch	12	12
S6	3-Position, on-off-on, paddle-handle toggle switch	12	12
S7	3-Pole, 12-position, nonshorting steatite switch	1	0
S10	3-Pole, 6-position, ceramic rotary switch	0	3
L1	NE-51 lamp (miniature neon-glow indicator lamp)	1	1
L2	Light sockets: miniature bayonet, 13-V lamp	12	0
F1	Fuse holder: standard 1/4- by 1 1/4-in fuse, 1/2-A	1	1
F2	Fuse block for 1/4- by 1 1/4-in fuse, 1/4-A	3	3
C1	0.1-μF, 600-V capacitor	0	1
T1	Stancor P8609 transformer: 115-V, 60-Hz primary; 26.8-V, 1-A CT secondary	1	1
T2	Transformer: 115-V, 60-Hz primary; 25.2-V, 1-A CT secondary	1	0
T3	Transformer: 115-V, 60-Hz primary; 25.2-V, 2-A secondary	1	0
F	Pamotor 115-V miniature fan, type 4500p	1	0
R8	500-Ohm, 2-W potentiometer	12	12
R9	10-Ohm, 2-W resistor	1	0
R10	1.2-Ohm, 0.5-W resistor	0	3
CrY	Relay: 24-V-d.c. coil, high-voltage contact	12	0
J1-8	Insulated banana jacks, half red, half black	38	30
DVM	James digital voltmeter JE 801, kit form (James, P.O. Box 822, Belmont, CA 94002)	2	0
M1	Milliamperemeter: d.c., mirror scale with ranges 0-15, 0-50, 0-150 mA; Centurion model 4-05 (Beede Electrical Inst. Co., Penacook NH 03301)	0	3
D11-17	1N 4003 1-A, 200-V _{RM} (wkg) diode	0	29
TWELVE-VOLT REGULATED POWER SUPPLY			
D1,2,4	1N 4003 1-A, 200-V _{RM} (wkg) diode	3	3
D3	1N 4742 12-V, 10-W zener diode	1	1
C2,3	1,000-μF, 35-V capacitor	2	2
C4	0.1-μF, 100-V capacitor	2	2

Table 7.—Parts list for the constant-current regulator, electronic components—Continued

Circuit esignation	Description	Quantity	
		Option 1	Option 2
TWELVE-VOLT REGULATED POWER SUPPLY—continued			
R3	10-Ohm, 10-W resistor	1	1
R4	1,000-Ohm, 0.5-W resistor	1	1
R5	160-Ohm, 1-W resistor	1	1
Q3	2N 3055 transistor (RCA)	1	1
CONSTANT-CURRENT REGULATOR			
Q1	DTS-710 transistor (Delco Electronics)	12	12
Q2	FMP5-5172 transistor (Motorola)	12	12
R1	2,200-Ohm, 2-W resistor	12	12
R2	270-Ohm, 2-W resistor	12	12
DVM POWER SUPPLIES			
D5-8	1N 4003 1-A, 200- V_{RM} (<i>wkg</i>) diode	8	...
D9-10	1N 4742 12-V, 10-W zener diode	4	...
C5-6	1,000- μ F, 35-V capacitor	4	...
R6-7	470-Ohm, 0.5-W resistor	4	...
A.C. POWER-CONTROL SAFETY-INTERLOCK CIRCUIT			
S8	Double-pole single-throw toggle switch	1	1
S9	Miniature magnetic reed switch	1	1
Cr	Double-pole single-throw relay; 115-V-a.c. coil, 25-A contacts (Potter-Bumfield PR 7AYO)	1	1
L3	6-W, 115-V incandescent red lamp	1	1
F3	Fuse holder: standard 1/4- by 1 1/4-in fuse, 15-A	1	1
ELECTROPHORESIS SUPPORT STATION, CIRCUIT 1			
S11-16	Single-pole double-throw, on-on toggle switch	72	72
J9-16	Insulated banana jacks, half red, half black	96	96
J17	6-Contact printed-circuit board edge connector	12	12
ELECTROPHORESIS SUPPORT STATION, CIRCUIT 2			
S17-20	Single-pole double-throw, on-on toggle switch	12	12
J18-25	Insulated banana jacks, half red, half black	24	24
ELECTROPHORESIS SUPPORT STATION, CIRCUIT 3			
J26-33	Insulated banana jacks, half red, half black	24	24
MISCELLANEOUS			
...	Pointer knobs	26	26
...	15-Contact printed-circuit board edge connector for DVM's	2	0
...	Lug-type terminal strip	1	0
...	Bud box: 2 by 4 by 1.5 in	13	13
...	6-Contact printed-circuit board edge connector for LVPS	1	1
...	Photosensitized, one-side-only printed-circuit board for LVPS, 3 by 6 by 1/8 in	1	1
...	11-68012 Printed-circuit board heat sink	13	13
...	Photosensitized, one-side-only printed-circuit board for CCR, 6 by 6 by 1/8 in	3	3
...	6-Contact printed-circuit board edge connector for DVM power supply	2	0
...	Photosensitized, one-side-only printed-circuit board for DVM power supply, 4 by 4 by 1/8 in	2	0
...	Magnet: 3-in stir bar, for S9	1	1
...	25-Contact printed-circuit board edge connector for CCR	3	3

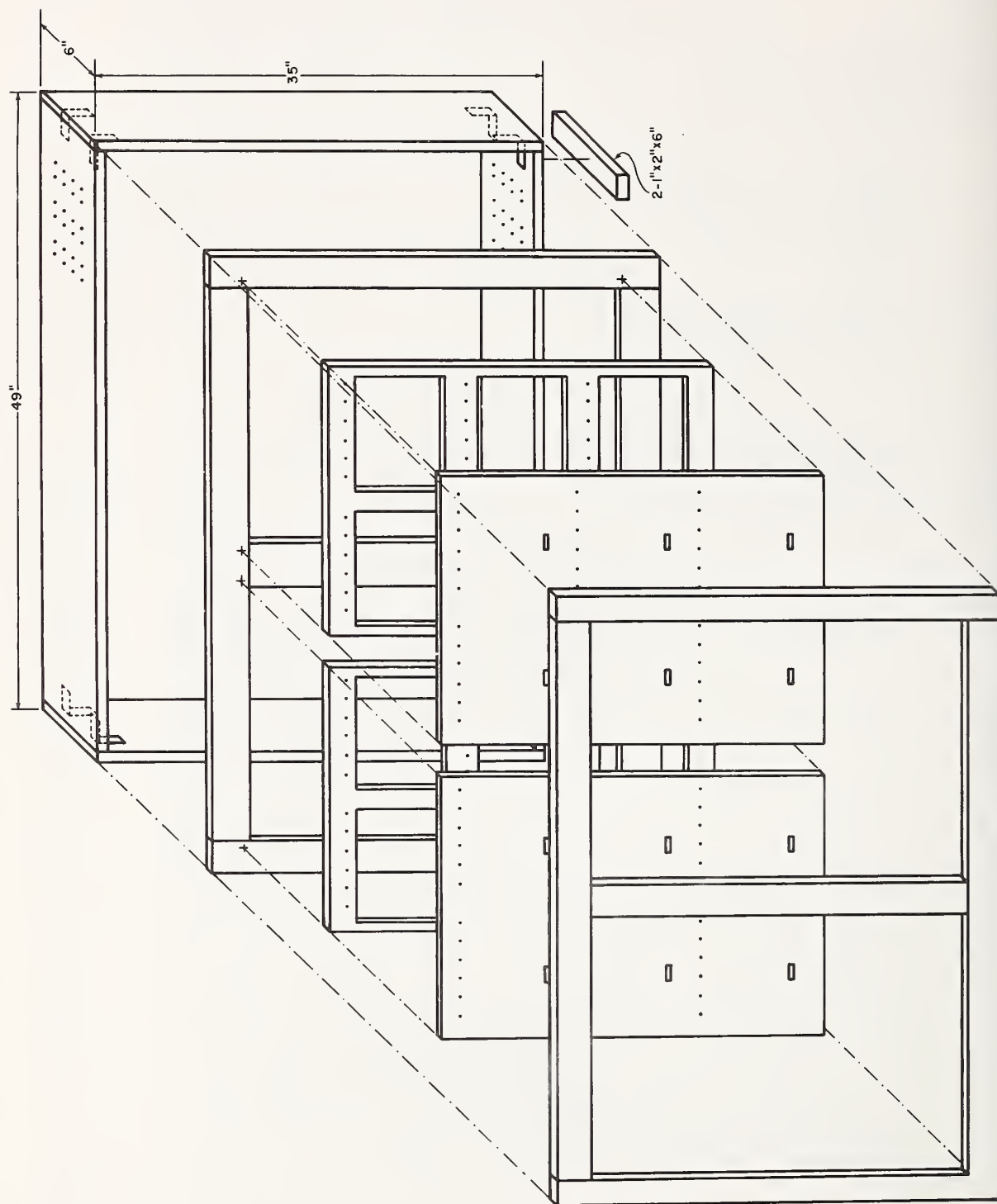


FIGURE 10.—Construction details of the electrophoresis support-station frame.

With regard to item 4 above, the versatility of the multifunctional electrophoresis system can be improved by the addition of extra circuitry to the support station. The circuitry shown in figure 11 allows four of the stations to be connected in series with each power supply. By use of the constant-current mode, each station can be turned off independently without affecting the current to the remaining stations. The current indicated on the meter on the front of each power supply is the actual current flowing through the cells connected to the power supply that is in the on position. As the cells are turned off, the power-supply voltage drops as that cell is bypassed. This arrangement also allows for constant-voltage and constant-power runs, the main advantage of this circuitry. An additional circuitry that has proven helpful is a direct contact from each station to the power supply. This contact is done in groups of three stations with separate leads to each of the four supplies.

With the above circuitry, any combination of runs is possible, from 1 disk gel, constant current,

to 12 vertical-slab gels, constant power, and any combination.

Construction details

Frame.—The support-station frame is constructed as pictured in figure 10. The $\frac{1}{4}$ -in holes for the electrical wiring and for the coolant-distribution lines are drilled according to the specifications in figure 12, after the frame is completely assembled, as are the $1\frac{1}{4}$ - by $\frac{1}{4}$ -in slots. Also, after the frame is assembled, the shelf supports are attached as illustrated, and the shelves are constructed as in figure 13 for the disk-gel and vertical-slab-gel baths to be described. Routinely kept on hand are a number of shelf brackets that may be cut to various sizes for shelves used for other equipment. The two notches on either end of the shelves serve to make them flush with the face of the support station, and the shelf brackets fit into the grooves, forming a sturdy support.

Illumination.—The 12 stations are illuminated
(Continued on page 22.)

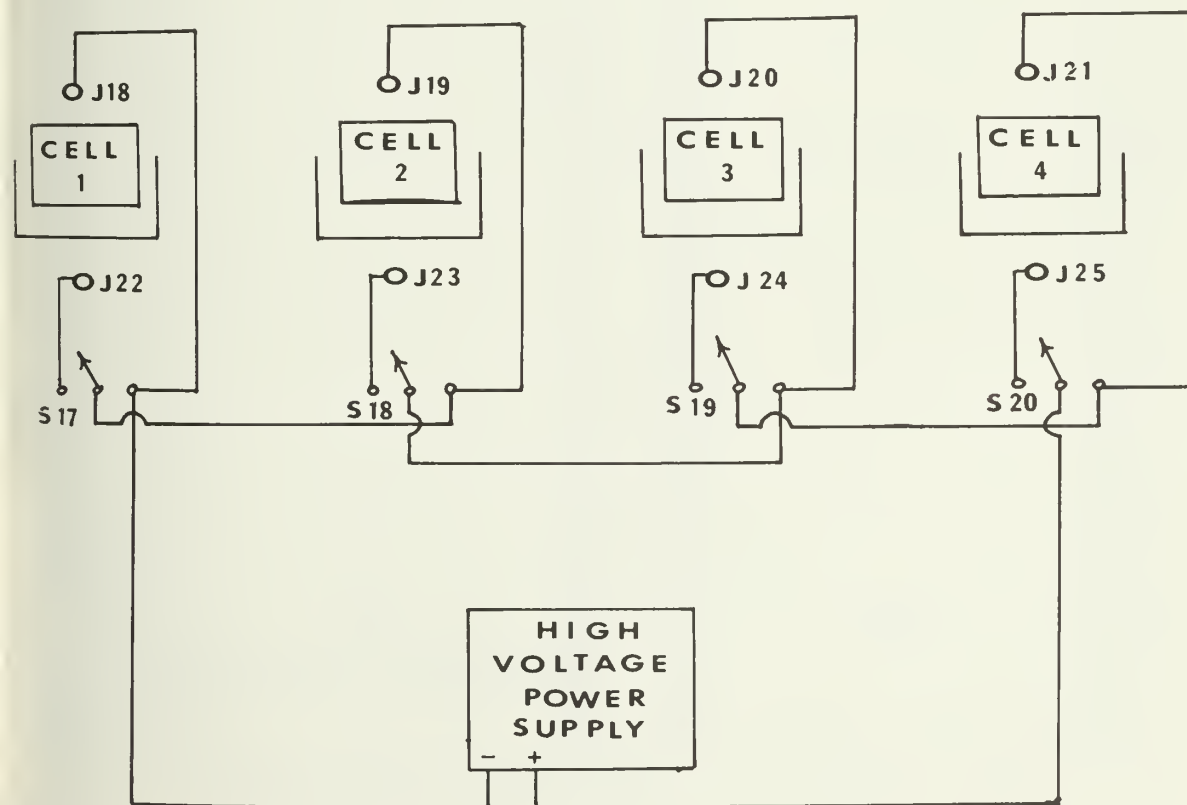


FIGURE 11.—Optional circuitry for the electrophoresis support station (used in place of the constant-current regulator for certain applications). Note: One of three identical circuits.

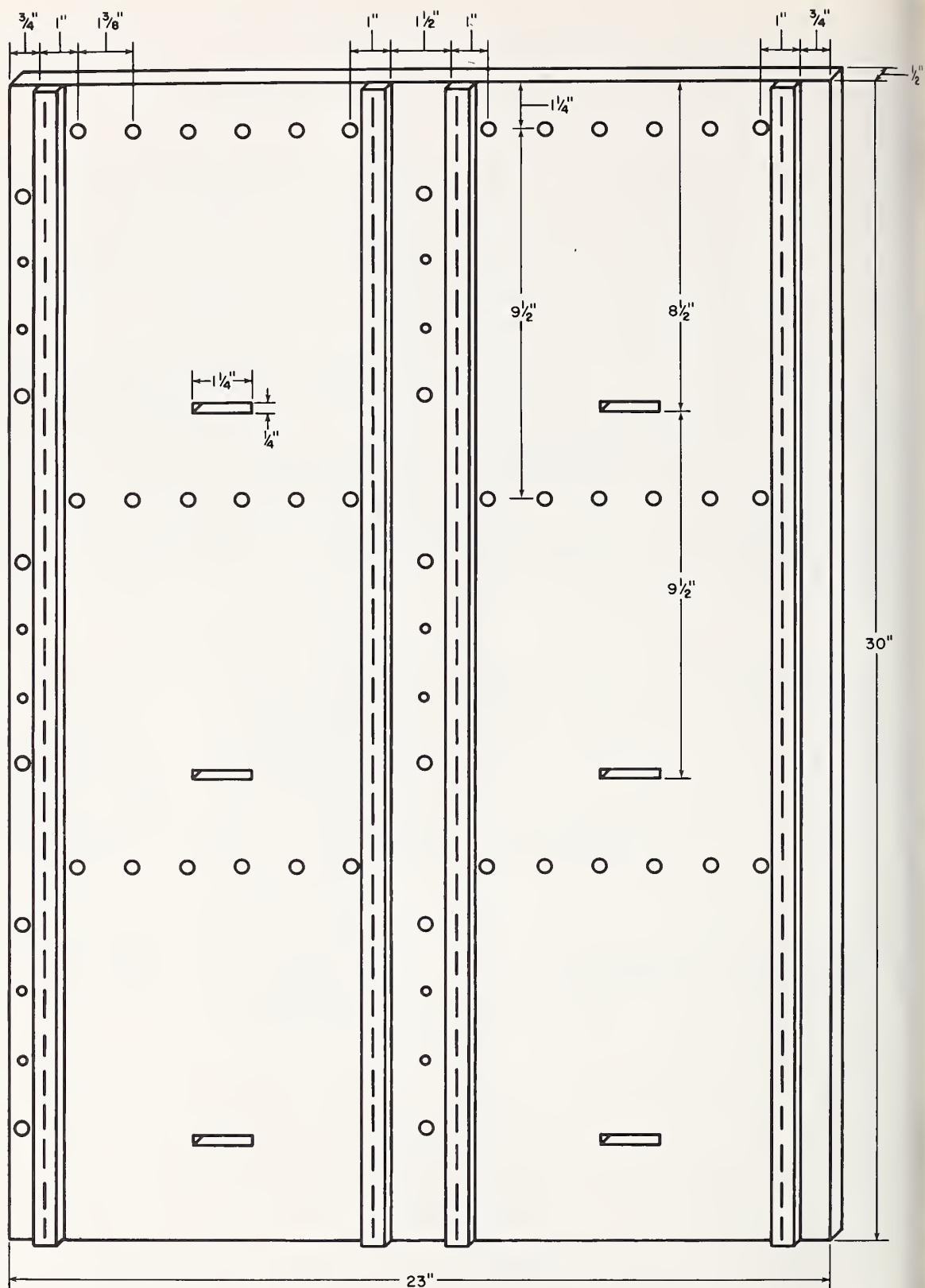


FIGURE 12.—Construction details of the translucent face of the electrophoresis support station. Note: Only half shown; other half is identical.

by means of 6 daylight fluorescent lights mounted in back of the front panel of the support station. The two lights for each of the three tiers of stations are positioned 1 in from the top and bottom of the illumination ports, respectively, and about 3 in behind them. The lights are centered from both ends, and the electrical connections are attached to two strips of acrylic plastic fastened to the top and bottom of the support frame. The ballasts for the lights are attached to the floor of the support frame.

Circuit wiring.—Appropriate lengths of lead wire are soldered to 72 insulated banana jacks and fed through the $\frac{1}{4}$ -in holes from the front side, alternating red and black, for each of the 12 stations (the alternating configuration was chosen to avoid possible cross wiring when disk-gel electrode leads are attached). The jacks are permanently fastened to the frame with a drop of Kodak 910 adhesive. From the back side, silicon rubber is used to fill the holes, firmly attaching the wire leads and also insulating them from the rest of the apparatus. Lead wires of appropriate length are attached to the six-contact printed-circuit board edge connectors (table 7). In a similar fashion, the wires are fed through the slots in the frame face, the edge connectors are permanently affixed with nylon bolts, and the lead terminals are coated with silicon rubber.

The groups of six leads from each station are wrapped into bundles, attached to the wooden framework with plastic clamps, and fed through the appropriate 1-in hole located on the vertical crossmember of the frame. The on-off switches (S11–16) are mounted into bud boxes (6 in each of 6 boxes, 12 in each of 3 boxes), and the wire leads from each of the 12 stations are attached to their appropriate terminals. The bud boxes are attached to the face of the support frame. The lead wires from the bud boxes (two for each station) to the constant-current regulator are attached to jacks (J9, J10, fig. 2) located at the top (for cooler installation) or bottom (for cart installation) of the support station and are permanently fastened to the frame. The electrical wiring of the other circuits of the support station is straightforward as described, except that the switch and insulation must be of a greater capacity than the maximum applied voltage. In this case, number-18 gage, 5,000-V-test probe wire was used. Also, be sure that all exposed metal parts (lamp ballasts, switchbox housing, brackets, and so forth) are grounded.



FIGURE 14.—Working setup of the multifunctional electrophoresis system, option 1.

Coolant-distribution lines.—Through the $\frac{1}{4}$ -in holes on the vertical crossmembers of the support station are passed $\frac{1}{8}$ -in-i.d. by $\frac{1}{4}$ -in-o.d. Tygon tubing long enough to allow their attachment to the vertical sides and bottom of the frame in the same manner as described for the electrical wiring. The tubing is cemented in place where it passes through the face of the support station, and a polyethylene nipple is attached to each. A nipple is also attached to the other end, which protrudes through the left-hand bottom of the frame. Before the tubing is connected to the frame, each group of six tubes is first wrapped with insulating material to retard heating of the circulating coolant. The support station can either be placed in the refrigerated cooler or moved around on the cart. The support station need only be bolted in place on the cart and the necessary connections made to the coolant-valving system and to the constant-current regulator.

If tubing of a larger diameter is desired throughout the cooling system, the appropriate modifications should be made at the start, because the major components, located in the cart, will be inaccessible after construction.

If cart mounting is chosen, the back of the



FIGURE 15.—Working setup of the multifunctional electrophoresis system, option 2. Insert: Installation of faucets to external wall of cooler and attachment of self-priming pump with remote on-off cable.

support station can be fitted with shelving. This space can be used for storage or to prepare gels with photopolymerization, while electrophoresis is progressing up front.

Refrigerated-cooler installation

Figures 14 and 15 show the arrangement of the major components of two electrophoresis systems when mounted in 40-ft³ refrigerated coolers, and is self-explanatory. The master power switch (S8) activates the safety-interlock and illumination system of the electrophoresis support station. The power light (L3) remains on when the main power switch is on and the cooler doors are closed. When the cooler doors are opened at least one-fourth in, all power is shut off and the light goes off because of the tripping of switch S9. The power-distribution control box, consisting of two four-place receptacles (five controlled by the safety circuit), sits behind the high-voltage power supplies.

Connections between the regulator and the support station are made through ports drilled through the cooler, either from the top or the side, depending on the configuration used. The support station sits on a wooden platform within the cooler. Underneath this platform are four 22-l carboys used for buffer storage. Four Chemtrol faucets can be installed through ports in the side of the cooler and connected to the carboy faucets by means of quick disconnects. Quick disconnects can also be mounted on the outlet side of the faucets for the attachment of a self-priming pump, as shown in the insert of figure 15. A 4-ft-long pushbutton on-off cable attached to the pump allows the flow to be controlled from a remote position when the electrophoresis baths are being filled.

The cooler sits on a 12-in-high wooden platform to raise the enclosed support station to a comfortable height for observation of all 12 stations during an electrophoretic run. The cooler is normally kept at 5° C to keep the buffer in the reservoirs and the running gels from overheating.

Cart installation

The multifunctional electrophoresis system can be mounted on a cart. A cart and an external cooling system was designed to allow for individual cell cooling and storage of the ancillary electrophoresis equipment.

The cart (fig. 16) measures 49 in long, 32 in wide, and 35 in tall and is mounted on 2-in lockable casters. The power supplies and the constant-current regulator are sunk flush with the top and are supported by an open shelf within the cabinetry, allowing air to circulate freely around them. (The plans in figure 16 were designed to use three ISCO 490 power supplies and the constant-current regulator, option 2; if different instruments are used, the dimensions will have to be modified to accommodate them.) The coolant-valving station (described later) is also sunk into the top. A hinged, slanting acrylic-plastic door covers these units to protect them from accidental spills.

The cart is also equipped with two 20- by 30- by 5-in drawers and one 20- by 30- by 8-in drawer located in the front, providing sufficient storage space for most of the ancillary equipment. Three 23- by 23-in doors, one in front and two in the back, allow complete access to the interior of the cabinet. Inside is space for a refrigerated circulating bath and sufficient room for buffer storage. Directly under and to the rear of the coolant-valving station is a double-walled box containing the constant-flow manifold and the return manifold of the coolant system (described later). On the left-hand sidewall of the box are located the 12 supply and 12 return nipples of the cooling unit. When the electrophoresis support station is in place, a short piece of Tygon tubing is attached to each nipple, and the other end of the tubing is attached to the appropriate nipple on the bottom of the support station, which is accessible through the rectangular panel on the top left side of the cart. Likewise, the electrical connections between the support station and the constant-current regulator are made through the panel at the top right side of the cart.

An electrical strip with eight three-prong outlets is located on the shelf that holds the power supplies. The refrigerated circulating cooler, the illumination system of the support station, the power supplies, and the constant-current regulator are plugged into these. A single three-pronged plug emerges from the side of the cabinetry for wall-plug insertion to supply power to the entire unit. The cart is also equipped with removable, sliding Plexiglas doors that cover the front of the electrophoresis support station when it is in place (fig. 17). A safety-interlock system is attached

(Continued on page 28.)

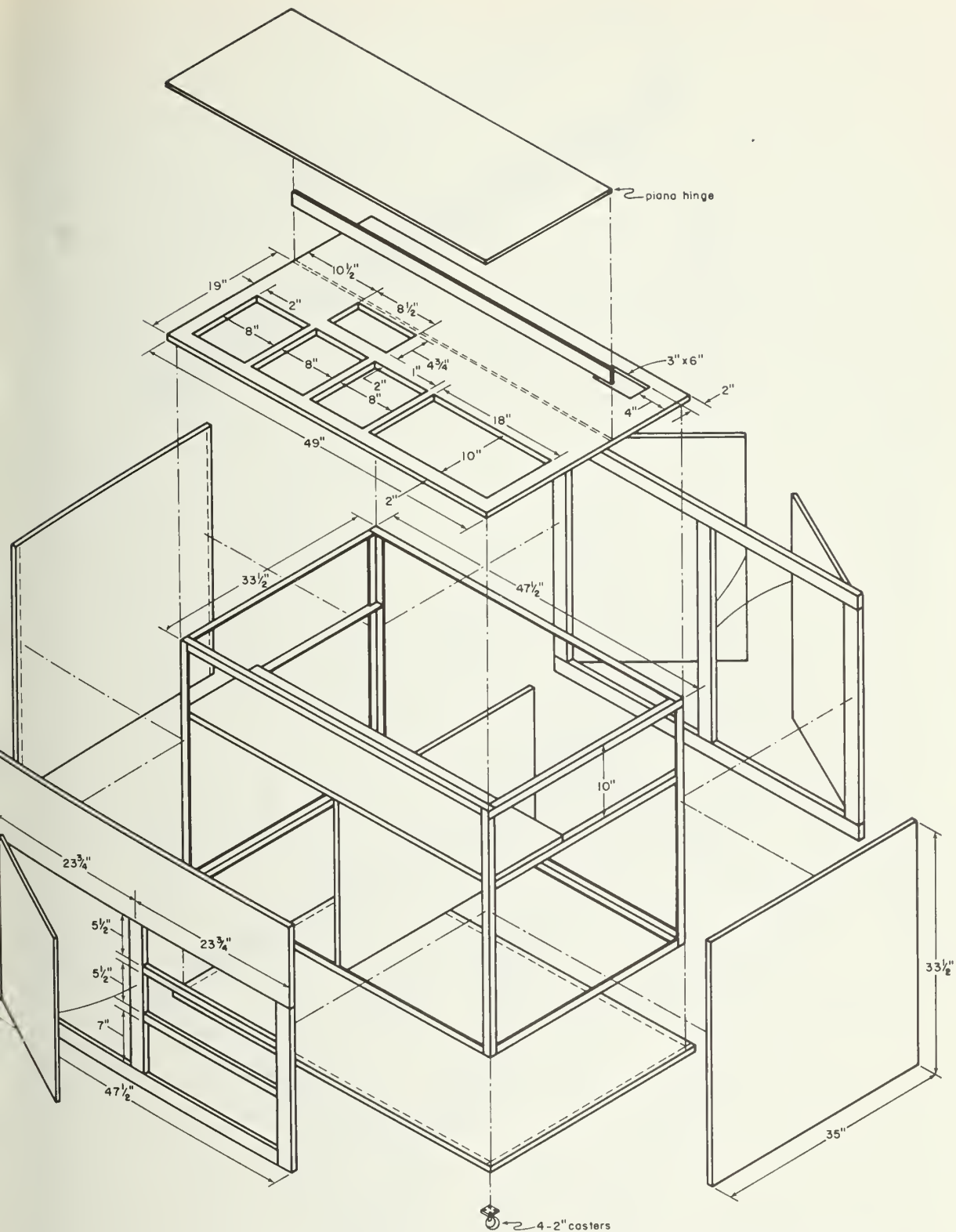


FIGURE 16.—Construction details of the cart used for mounting the multifunctional electrophoresis system.

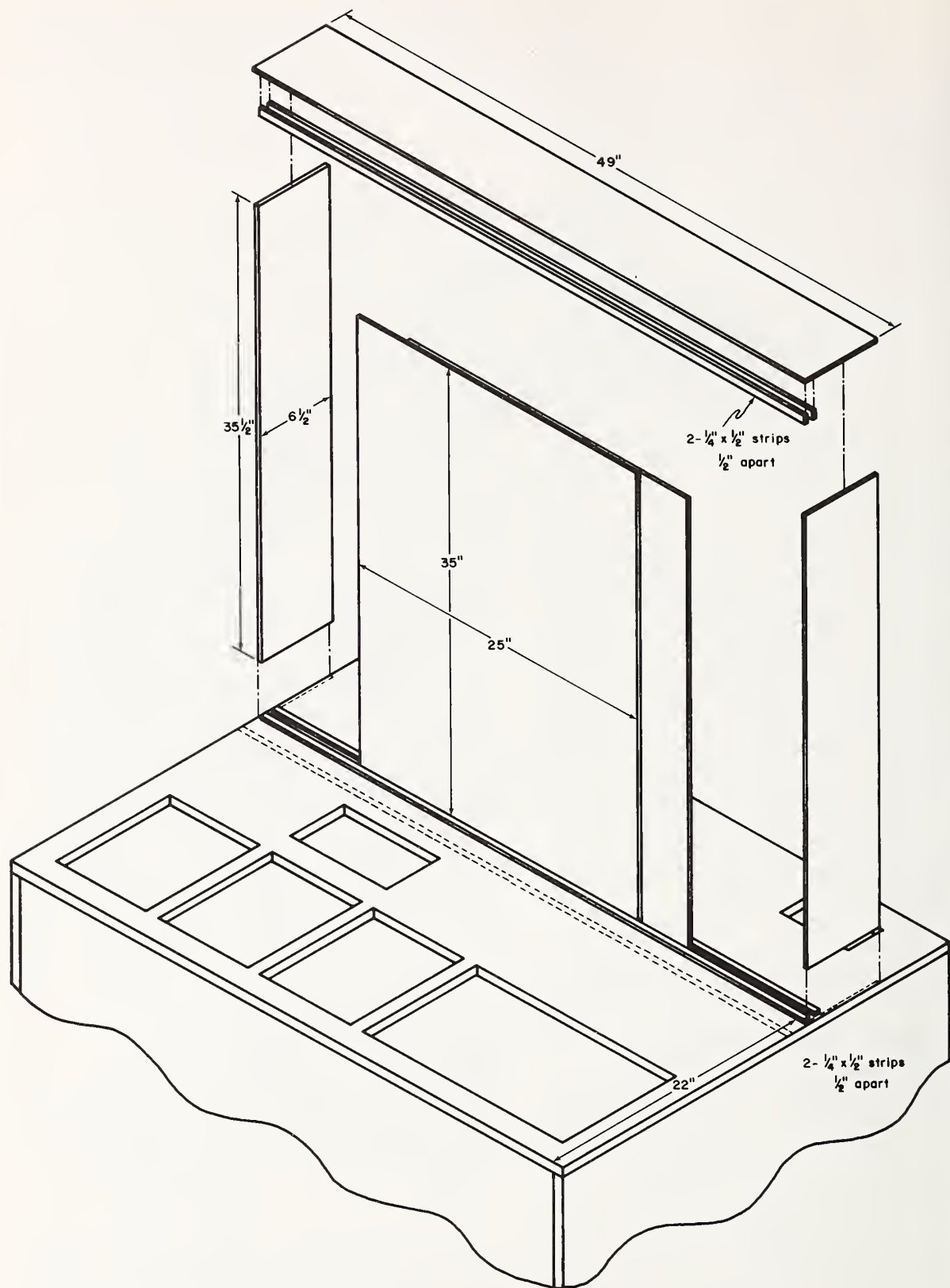


FIGURE 17.—Details of the cart sliding doors that cover the front of the electrophoresis support station for operator safety.

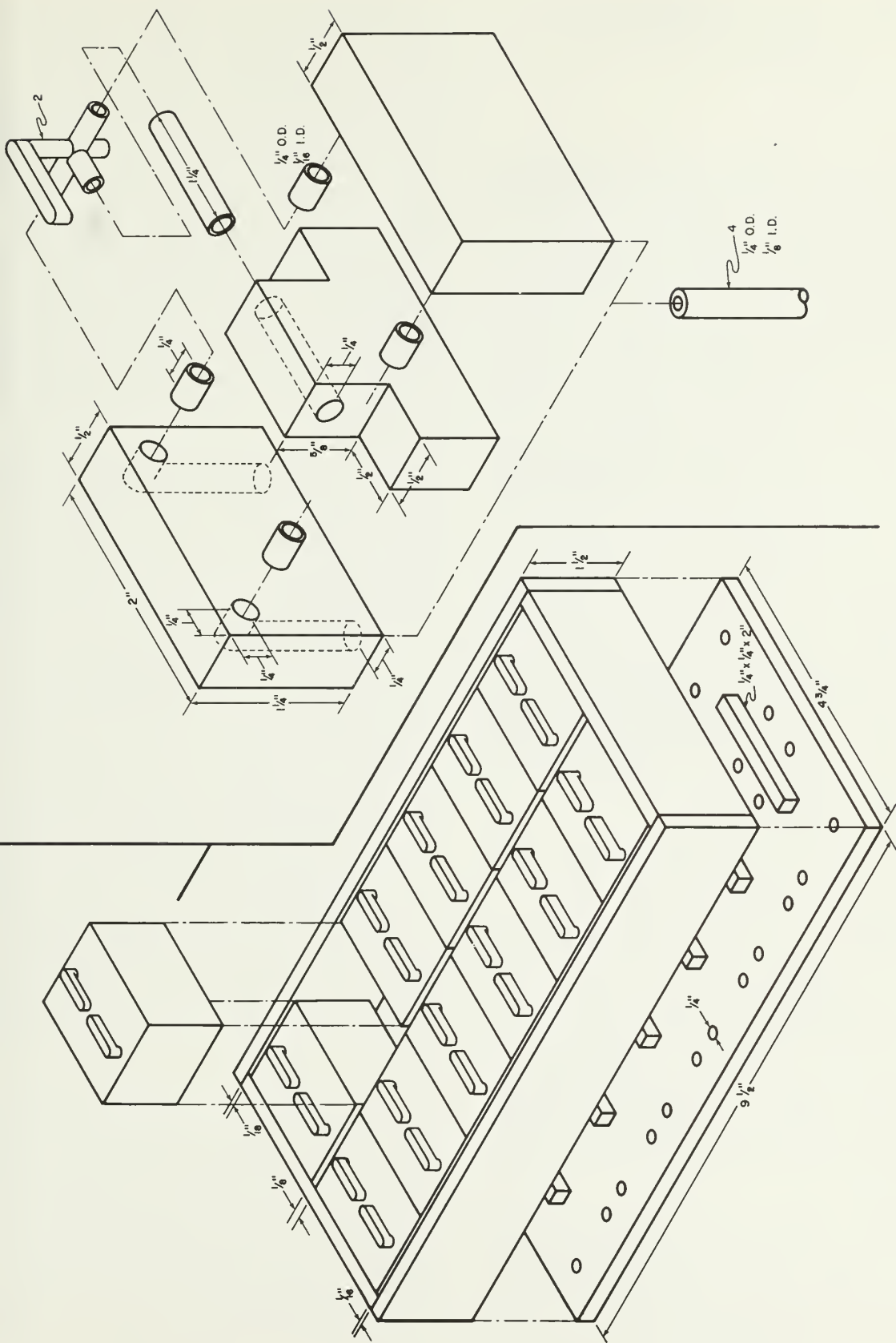


FIGURE 18.—Construction details of the coolant-valving station.

so that when a door is opened, the power to the power supplies and the constant-current regulator is turned off.

CART COOLANT SYSTEM

Coolant-valving station.—The coolant-valving station consists of 12 sets of three-way stopcocks that allow coolant to be pumped through an electrophoresis cell or to bypass it. The construction details are given in figure 18. The three arms of the stopcocks are first cut with a razor blade to a length of one-fourth in. A $\frac{1}{16}$ -in drill bit is used to enlarge the holes in the valve, and care is taken not to puncture the closed port position. After a $\frac{1}{4}$ -in hole is drilled through the center acrylic support piece, the odd arm of the stopcock is forced into one end of a 1-in-long by $\frac{3}{16}$ -in-i.d. by $\frac{1}{4}$ -in-o.d. piece of Tygon tubing. The hole in the support piece is lubricated with acrylic solvent and the tubing forced through until it is flush with the other end. The second stopcock is forced into this end (be sure that the stopcock arms are at least one-eighth in into the support piece and that the two sidearms are at a 90° angle to the top of the support piece). The acrylic solvent forms a tight, permanent seal between the tubing and the acrylic block. Make sure that the top of the drilled $\frac{1}{4}$ -in hole is no more than one-eighth in from the top of the support piece. If it is more than this, the handle of the stopcock will not turn freely.

After the stopcocks are attached, a $\frac{1}{4}$ -in-long by $\frac{3}{16}$ -in-i.d. by $\frac{1}{4}$ -in-o.d. piece of Tygon tubing is slipped over the remaining arms of the stopcocks and forced flush with the Tygon tubing on the odd arm. The two end acrylic pieces are then attached to the middle piece by forcing the arms of the two stopcocks into the $\frac{1}{4}$ -in holes. The tubing is again cemented to the acrylic with solvent, and the two side pieces are cemented to the middle acrylic piece. A vise is used to hold all three pieces together until the bond between them is made. After all 12 valving systems are constructed, they are tested for leaks by pumping water through them (under pressure) in both flow positions.

A $\frac{1}{4}$ - by $\frac{1}{4}$ - by 2-in acrylic strip is cemented to the bottom of each pair of valves, as shown in figure 18. Leave a $\frac{1}{8}$ -in space between the ends. An acrylic box is fashioned from $\frac{1}{4}$ -in stock that has an inside diameter of $4\frac{1}{4}$ in wide by 9 in long by $1\frac{1}{2}$ in high. The six groups of valving systems

are placed into the box so that a $\frac{1}{16}$ -in gap exists at each side. Then forty-eight $\frac{1}{4}$ -in holes are drilled into the bottom of the box directly opposite the holes in the bottom of the valving blocks, and an appropriate length (about 18 in) of $\frac{1}{8}$ -in-i.d. by $\frac{1}{4}$ -in-o.d. Tygon tubing is passed through the 48 holes in the bottom of the valving blocks and cemented in place. These tubes are passed through the floor of the box and the valves are cemented to the box by means of the $\frac{1}{4}$ - by $\frac{1}{4}$ - by 2-in acrylic supports. Self-leveling silicon rubber is used to fill the box, leaving just enough room for the handles on the valves to be free to turn (the silicon rubber does not stick to the stopcocks). After the silicon rubber hardens, the coolant-valving station is completely solid and watertight.

Constant-flow manifold.—The constant-flow manifold is designed to allow the coolant (under pressure) to be passed equally through the 12 outlets (fig. 19). The manifold consists of a $\frac{1}{2}$ -in-thick by $2\frac{3}{4}$ -in-i.d. by 4-in-o.d. ring of acrylic with 12 equidistant holes around the perimeter. A $\frac{1}{8}$ -in hole is drilled through the ring at the 12 positions, and a $\frac{1}{4}$ -in hole is drilled over the top of these halfway through the ring. Using $\frac{1}{8}$ -in-i.d. by $\frac{1}{4}$ -in-o.d. by $\frac{1}{4}$ -in-long pieces of Tygon tubing, a nipple connector is placed into the $\frac{1}{4}$ -in hole and forced into the $\frac{1}{8}$ -in hole (the fittings should protrude about $\frac{3}{8}$ in beyond the 4-in ring). Two 4-in-diameter circles are then cut out of $\frac{1}{4}$ -in acrylic stock and cemented to each face of the ring to form a closed system. Twelve 1-in-diameter circles are fashioned from $\frac{1}{2}$ -in acrylic stock. Through the center of six of these, a $\frac{3}{8}$ -in hole is drilled. One of these rings is attached to the center of one of the 4-in-diameter faces, and a $\frac{3}{8}$ -in hole is drilled through it directly in line with that on the 1-in ring. A 4-in length of $\frac{1}{4}$ -in-i.d. by $\frac{3}{8}$ -in-o.d. Tygon tubing is permanently fastened through this hole flush with the interior face.

Return manifold.—The return manifold (fig. 20) is an elongated box made from $\frac{1}{4}$ -in acrylic stock that has internal measurements of $6\frac{1}{2}$ by 1 by 1 in. The top has 12 equally spaced nipple connections cemented in place by means of $\frac{1}{8}$ -in-i.d. by $\frac{1}{4}$ -in-o.d. Tygon tubing, as explained for the constant-flow manifold. The bottom is fitted with a 4-in piece of $\frac{1}{2}$ -in-i.d. Tygon tubing that fits flush with the bottom through a $\frac{5}{8}$ -in hole. Two of the 1-in-diameter circles from the previous

(Continued on page 32.)

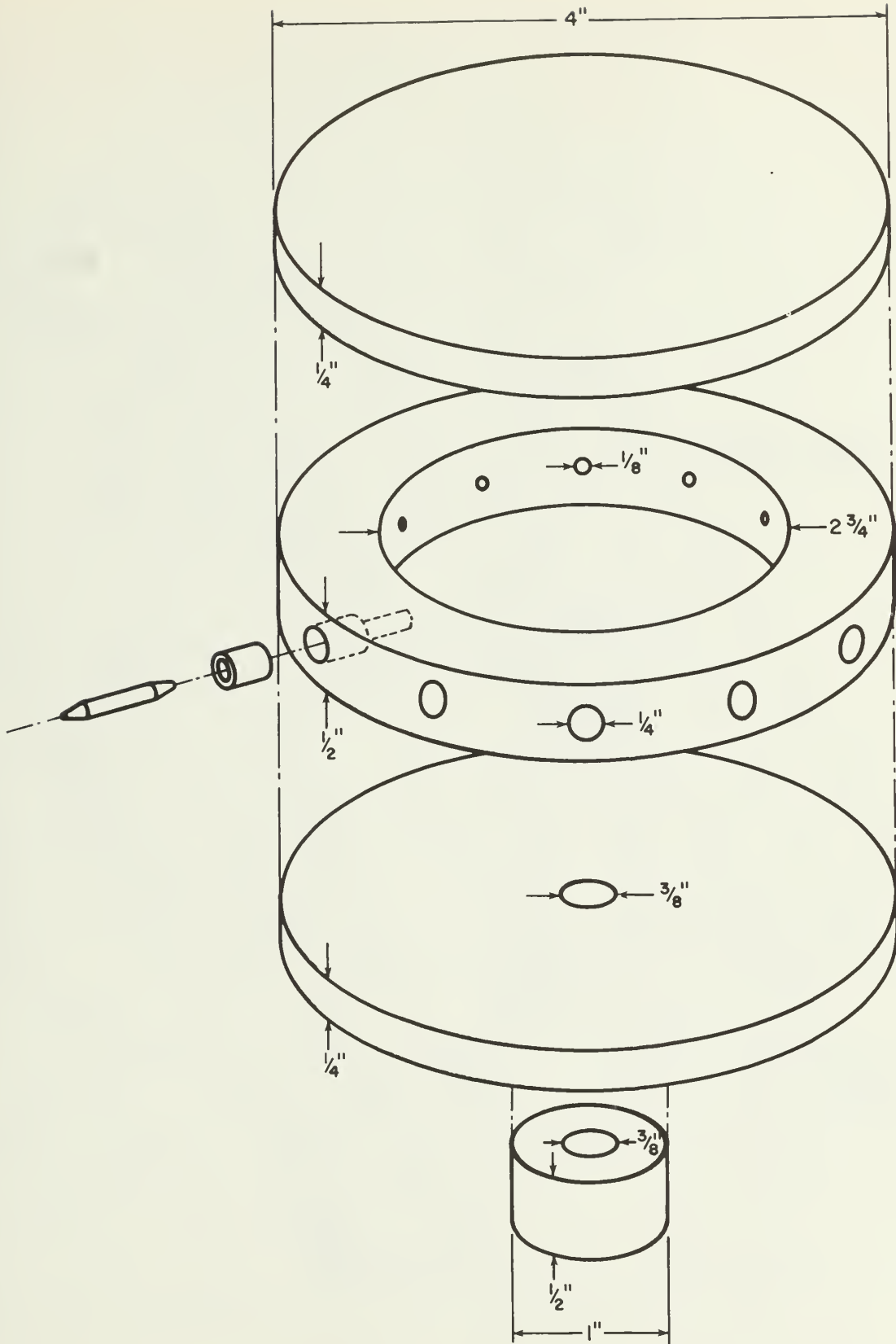


FIGURE 19.—Construction details of the constant-flow manifold.

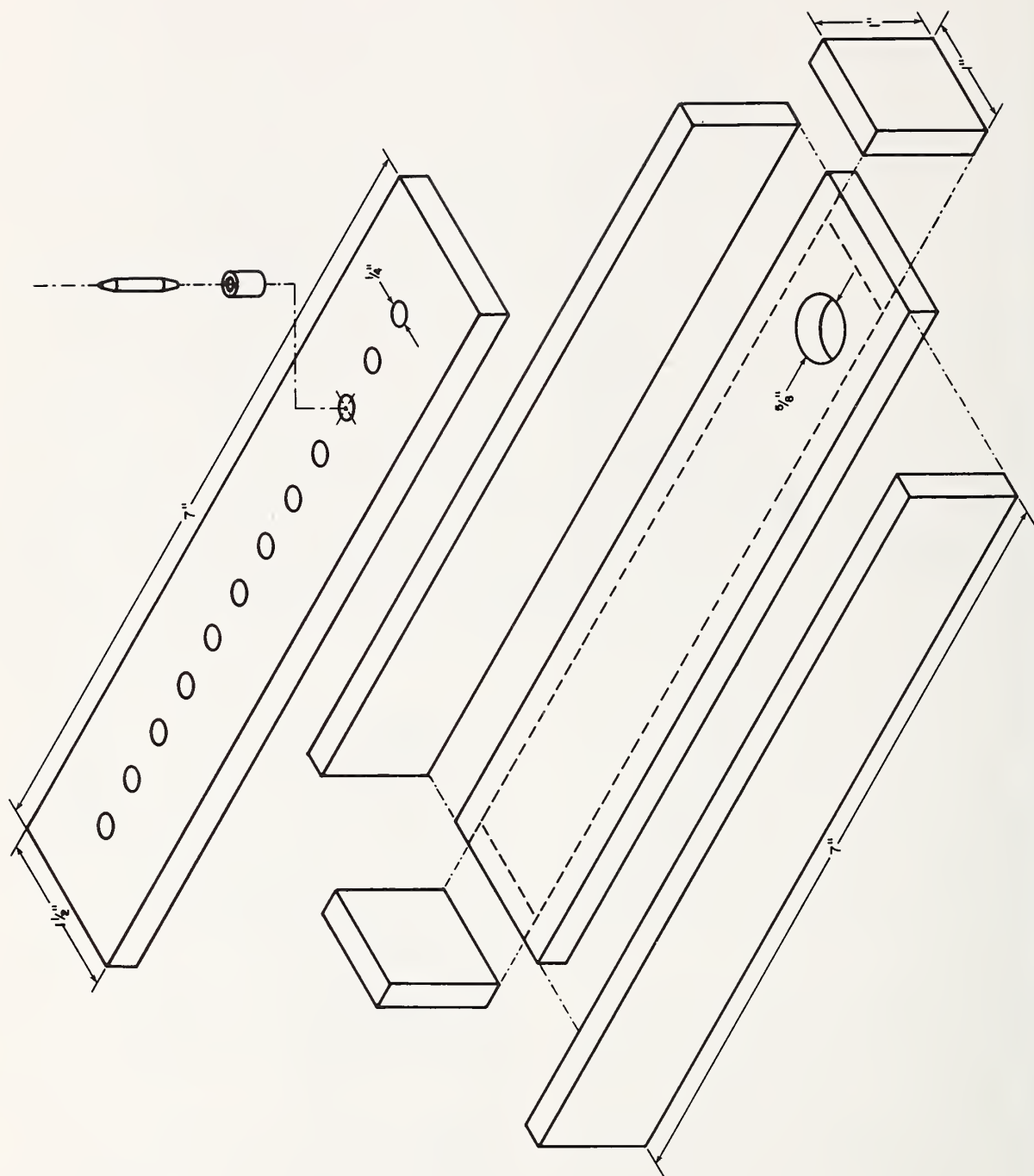
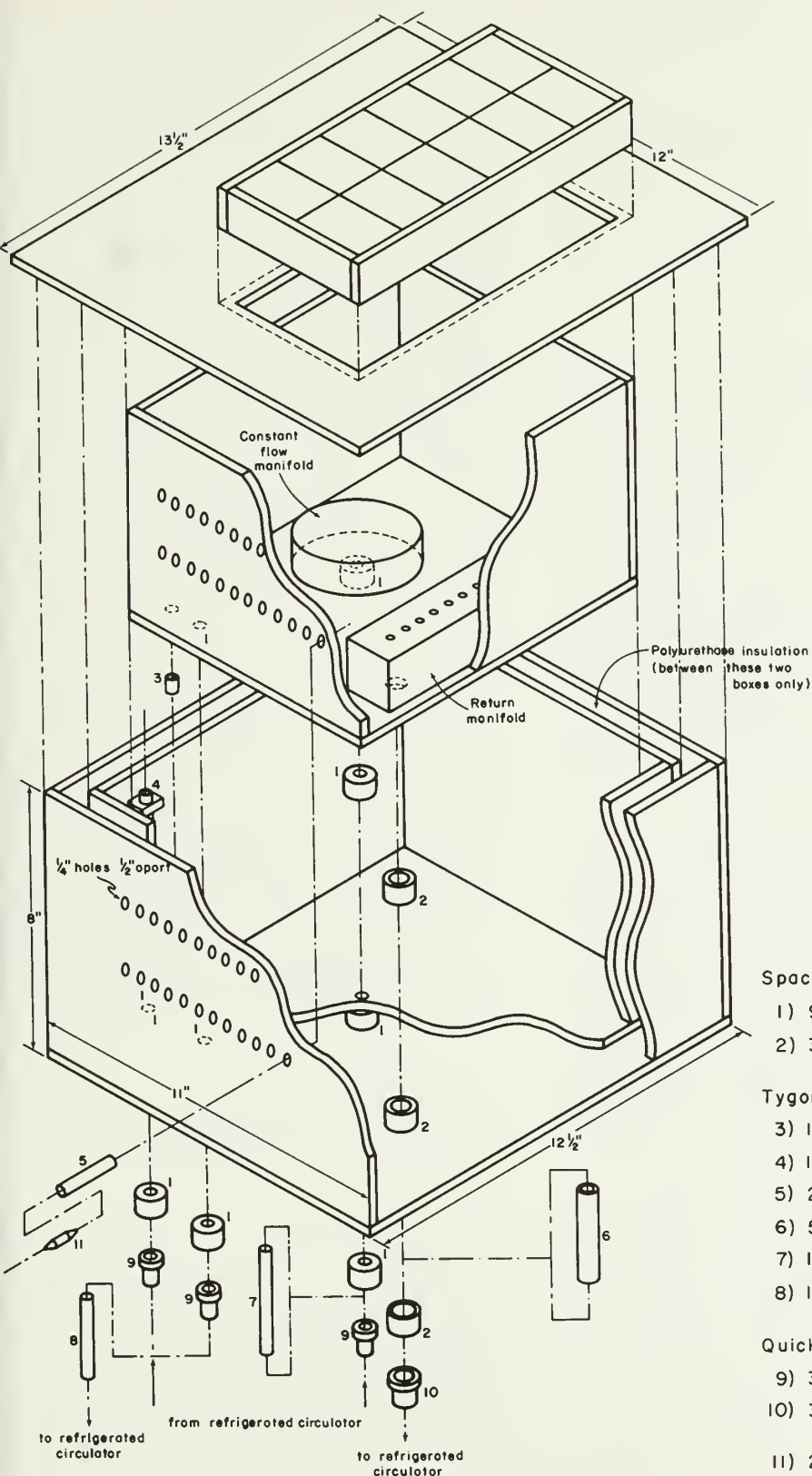


FIGURE 20.—Construction details of the return manifold.



Spacers (1/2" thick)

- 1) 9-1" O.D., 1/4" I.D.
- 2) 3-1" O.D., 5/8" I.D.

Tygon tubing

- 3) 1/4" O.D., 3/8" I.D., x 5/16"
- 4) 1/4" O.D., 3/8" I.D., x 8-1/4"
- 5) 24-1/4" O.D., 3/8" I.D., x 1-3/4"
- 6) 5/8" O.D., 1/2" I.D., x 2-1/2"
- 7) 1/4" O.D., 3/8" I.D., x 3"
- 8) 1/4" O.D., 3/8" I.D., x 2-1/2"

Quick disconnects

- 9) 3-1/4" - 3/8"
- 10) 3/8" - 1/2"

- 11) 24-NC-5 nipples

FIGURE 21.—Construction details of the double-walled coolant-system box.

section also have $\frac{5}{8}$ -in holes drilled through the center. The remaining 1-in acrylic circles are used to mount the appropriate quick disconnects, as explained in the next section.

Double-walled coolant-system box.—The purpose of the double-walled coolant-system box is to hold, in a leakproof environment, the coolant-valving station, the constant-flow manifold, and the return manifold, and to insulate these units so that the coolant from the refrigerated circulator does not warm appreciably on its way to the electrophoresis cells.

This structure is a box within a box and is constructed from $\frac{1}{4}$ -in acrylic stock. The top of the unit consists of a $13\frac{1}{2}$ - by 12-in piece of acrylic with a 9- by $4\frac{1}{4}$ -in opening located off center (fig. 21). The coolant-valving station is permanently sealed above this opening by means of its $\frac{1}{4}$ -in overhang. Attach the two ends of the inner box directly under the ends of the valving station. Drill the twenty-four $\frac{1}{4}$ -in holes into the left-hand end piece in the positions illustrated. Attach the $9\frac{1}{2}$ -in by 8-in bottom (with appropriate inlet and outlet holes) to the two ends. Position the constant-flow manifold with attached Tygon tubing above the appropriate $\frac{3}{8}$ -in hole, and permanently attach it to the bottom. Attach the return manifold above its $\frac{5}{8}$ -in hole in the same manner.

After the three units are permanently in place, make all tubing connections from the coolant-valving station to the constant-flow and return manifolds. The 12 tubes leading from the valving station to the constant-flow manifold should be of equal length. All connections should be made so that a gentle arc is formed between the units; crimps should be avoided to prevent excessive flow restriction. The connecting ends should butt against the Tygon tubing that holds the nipple connectors, and these should be permanently joined with cyclohexanone solvent.

Pass the 12 feed lines and 12 return lines through appropriate $\frac{1}{4}$ -in holes located on the left-hand end piece so that they protrude at least 3 in. Through one of the $\frac{3}{8}$ -in holes in the bottom, thread about 30 ft of $\frac{1}{4}$ -in-i.d. by $\frac{3}{8}$ -in-o.d. Tygon tubing into the lumen of the inner box in a spiral manner from bottom to top, around and between the tubing connections, and pass the free end through the other $\frac{3}{8}$ -in hole in the bottom of the inner box. Cut this off flush with the bottom (be sure to leave at least 3 in on the other end protruding through the bottom). Make sure

that none of the tubing is crimped and that all connections are tight. Check the system for leaks with running water. If everything is satisfactory, permanently attach the sides of the inner box. The entire lumen of this box is filled with self-leveling silicon rubber adhesive to hold the tubing permanently in place and to avoid the possibility of future leaking. The silicon should be poured in about $\frac{1}{2}$ -in depths over a number of days to allow complete curing.

Thread the appropriate circles of acrylic (from previous sections) through the protruding Tygon tubing ends, and permanently attach them to the inner and middle box bottoms. Position the coolant-return tube, permanently attach it as illustrated in figure 21, and complete the middle box, forming the $\frac{1}{2}$ -in coolant chamber. Position the acrylic circles and the four layers of $\frac{1}{8}$ -in-thick polyethylene insulation, and attach the outer box, as shown in figure 21. Affix the female end of the three $\frac{1}{4}$ -in to $\frac{3}{8}$ -in and the $\frac{3}{8}$ -in to $\frac{1}{2}$ -in quick disconnects to the appropriate protruding tube, as shown in figure 21. Affix the 24 nipple connections to the supply and return tubes. Throughout the construction of this unit, the tubing should be cemented to the holes with silicon adhesive to make them watertight and to insure that they will not slip. Fasten this unit to the underside of the cart top with wood screws by means of the $\frac{1}{2}$ -in flange on its top.

A schematic showing the flow pattern of the coolant system is presented in figure 22. In practice, if the water pressure has to be adjusted in any of the lines, an adjustable pinch clamp attached to the connection tubing outside the box can be used.

ANCILLARY ELECTROPHORESIS EQUIPMENT

The choice of electrophoresis systems and ancillary equipment that can be used in the complex reported in this paper is not critical. As mentioned in the introduction, almost any of the commercially available systems and many of the systems reported in the literature can be used with little or no modification. The systems described in the literature (Raymond 1962; Clarke 1964; Davis 1964; Allen and Moore 1966; Akroyd 1967; Besaw et al. 1972; Maurer and Dati 1972)

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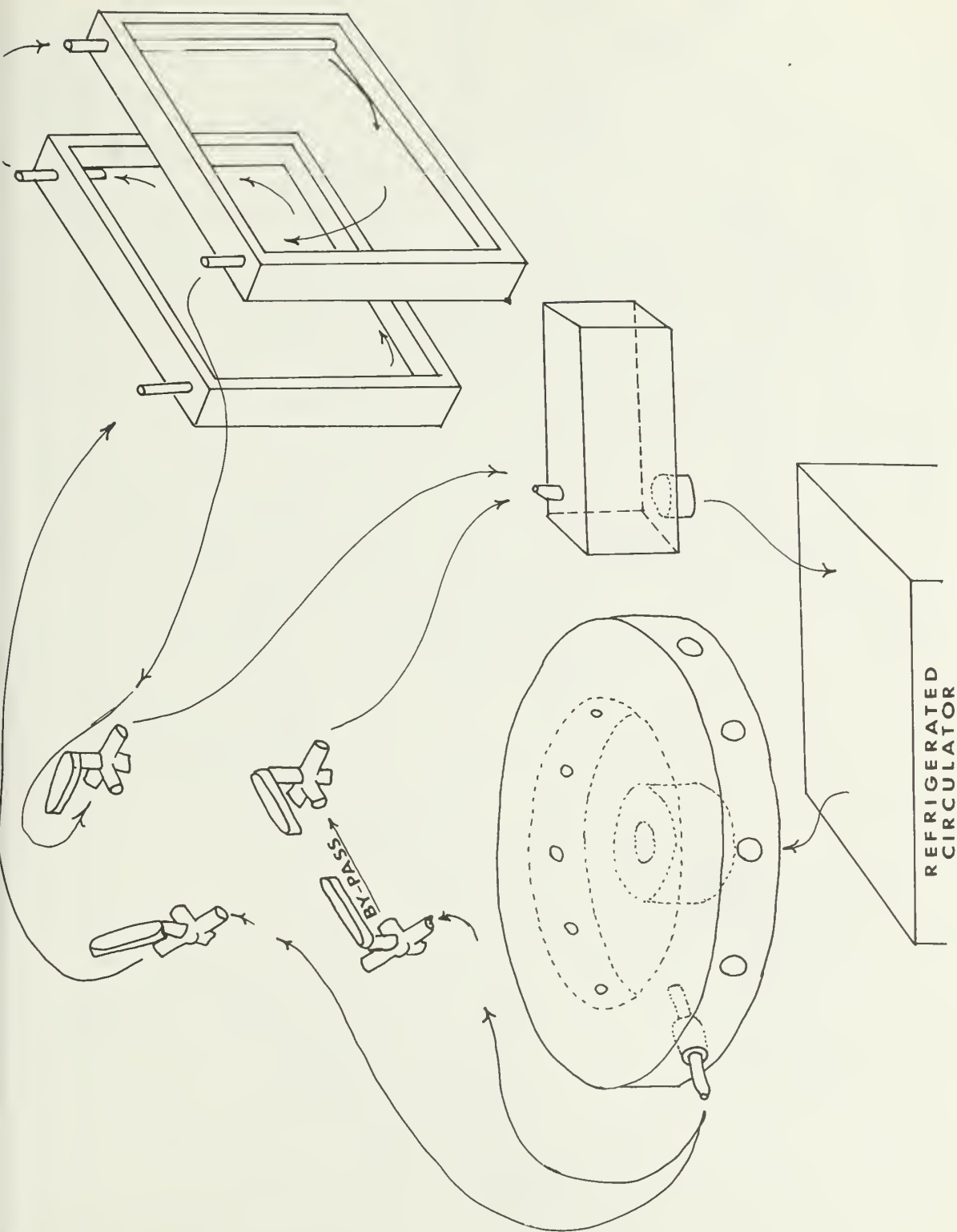


FIGURE 22.—Schematic showing the flow pattern of the coolant system.

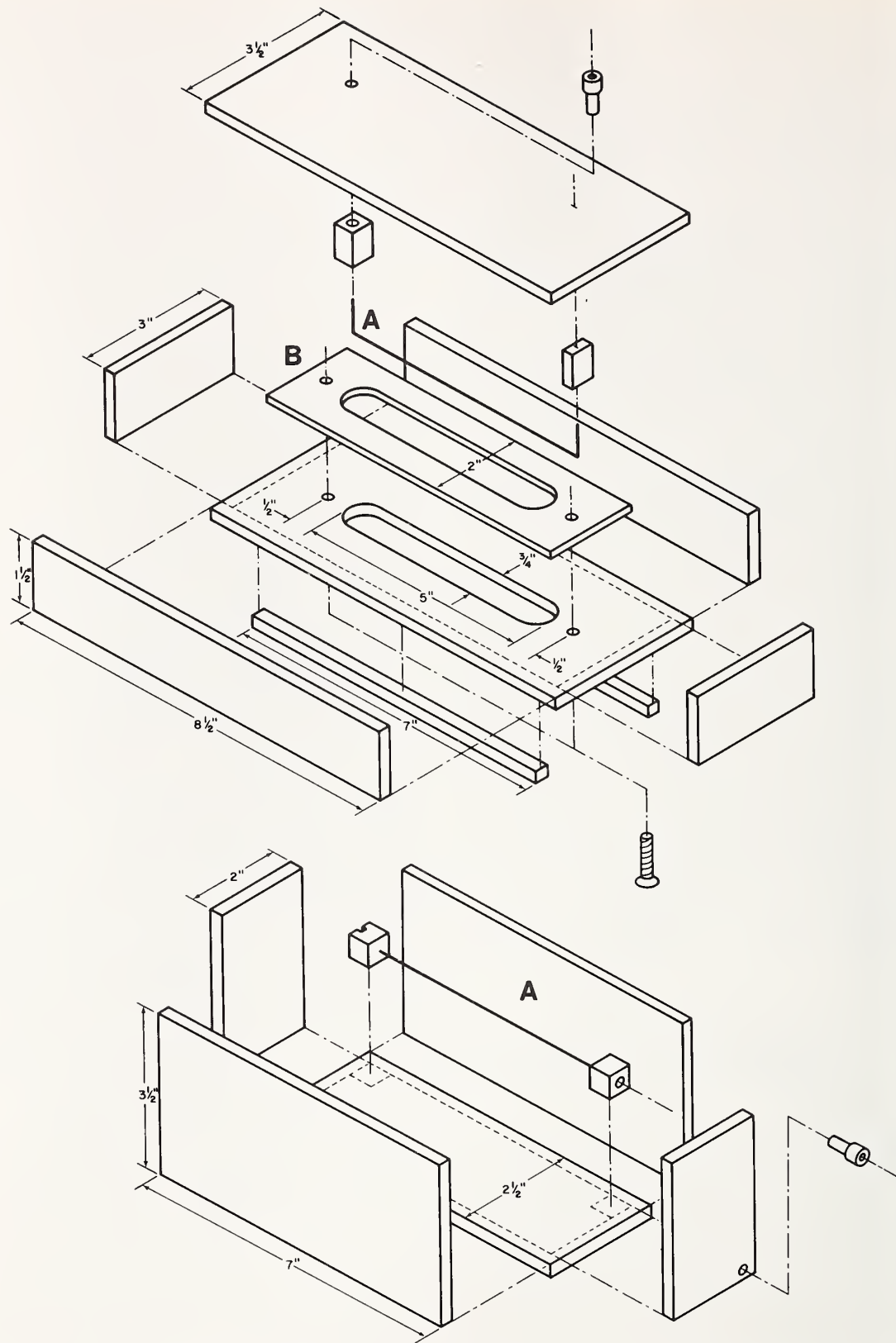


FIGURE 23.—Construction details of the upper and lower vertical-slab-gel baths. A, 24-Gage platinum wire electrodes; B, $\frac{1}{8}$ -in-thick polyethylene gasket and upper bath placement.

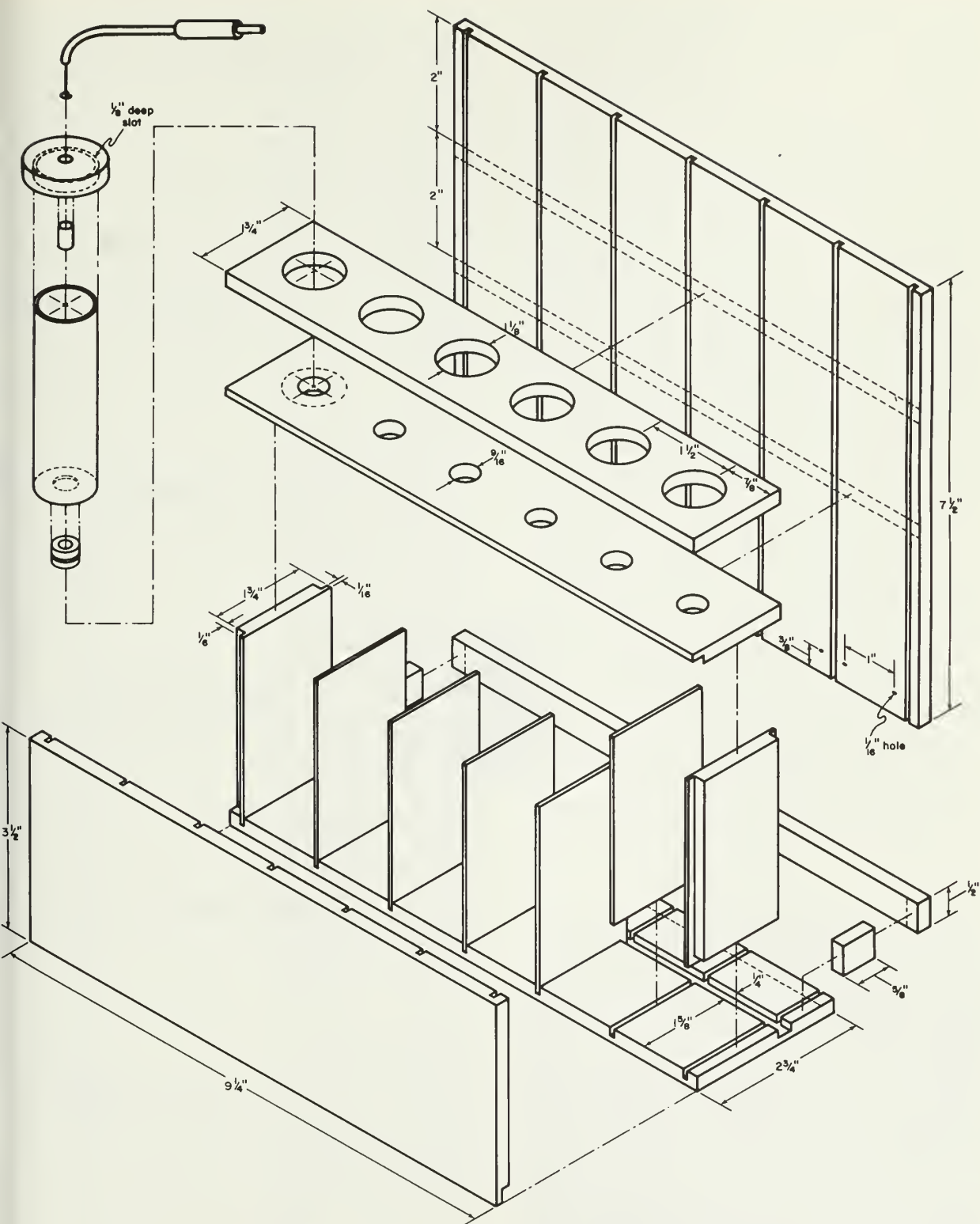


FIGURE 24.—Construction details of the upper and lower disk-gel baths.

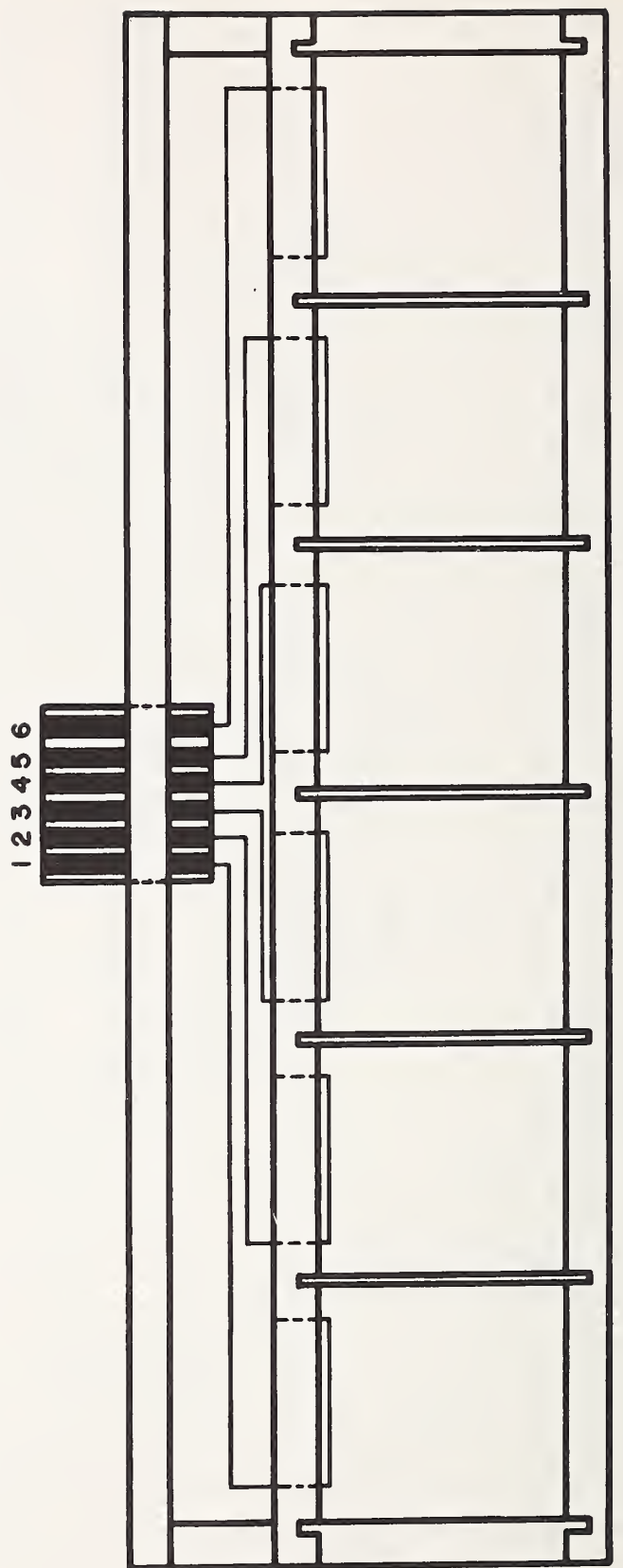


FIGURE 25.—Wiring diagram of the lower disk-gel baths.

and the commercial counterparts of six different companies have all been used successfully in conjunction with the multifunctional electrophoresis system, and especially the constant-current regulator at one time or another during the past 4 years. The choice of equipment should be made on the basis of what is suitable for a particular application. However, one should bear in mind the limitations of the constant-current regulator. If this module is to be used without modification, one must choose a system compatible with the power limitations or bypass this module and use the power supplies directly.

The following equipment best suited our needs.

VERTICAL-SLAB-GEL BATHS

Construction of the upper and lower gel baths used in this laboratory is straightforward as shown in figure 23, and is similar in design to that of Allen and Moore (1966). Some points in the design of these baths warrant comment. Some researchers (Woodworth and Clark 1967; Brewer 1970; Morris, C., and Morris, P. 1974) use either baffles or two buffer trays to keep the electrodes (fig. 23A) separated from the running buffers. This was necessary because of the changes in pH and conductance that occur near the electrodes, possibly affecting the gels. This is not a problem in the present setup, mainly because a large amount of buffer is used (300 ml in the upper bath, 600 ml in the lower bath), running times are usually less than 2 hours for electrophoresis, and a low current is normally applied. However, if running times are long or if currents higher than those suggested in this study are used, this point must be considered. If a baffle is necessary or desirable, install one in both the upper and lower baths by attaching Plexiglas barriers between the electrodes and the cells.

The molded nylon screw locations were chosen with versatility in mind. Not only can Ortec electrophoresis cells be used directly in these baths, but most of the precast commercially available gels can be simply incorporated into the system (see later). The use of $\frac{1}{8}$ -in-thick polyethelene as gasket material in the upper bath eliminates the need for the commonly used silicon grease as a sealant (fig. 23B).

DISK-GEL BATHS

The design of the disk-gel baths (fig. 24) is

similar to that of Besaw et al. (1972), which can be substituted. Unlike their unit, however, each column is supplied not only with its individual cathode buffer reservoir, but also an anode buffer reservoir, and each of the six pairs of baths is equipped with electrodes. The purpose of the individual baths is to use gels of different gel composition in the same apparatus while keeping the current constant through each, even though differences occur in gel and buffer resistance. This arrangement also makes the system more versatile and improves reproducibility. Most previous disk-gel systems use at least one common bath for a number of gels. During electrophoresis, one or more gels may run faster or slower than the others because of resistance differences between gels. To insure runs of the same length, current through some gels must be disconnected ahead of others. Depending on the equipment, this is either impossible to do or difficult to accomplish. Also, the current to the remaining gels must be adjusted if it is to be kept at a constant value throughout the run. The primary advantage of the apparatus described here is that the disk gel can be removed from the system without affecting the current through the rest. Herein lies also the major disadvantage of this system. Because the resistance of each cell is additive in this arrangement, the maximum drop throughout the six gels (using the constant-current regulator) is limited to 900 V. If the constant-current regulator is bypassed, the drop is limited only by the high-voltage power supply.

At low currents, pH shift resulting from electrolyte hydrolysis has not been a problem. How-



FIGURE 26.—Holding stand for 72 upper electrodes used with the disk-gel baths.

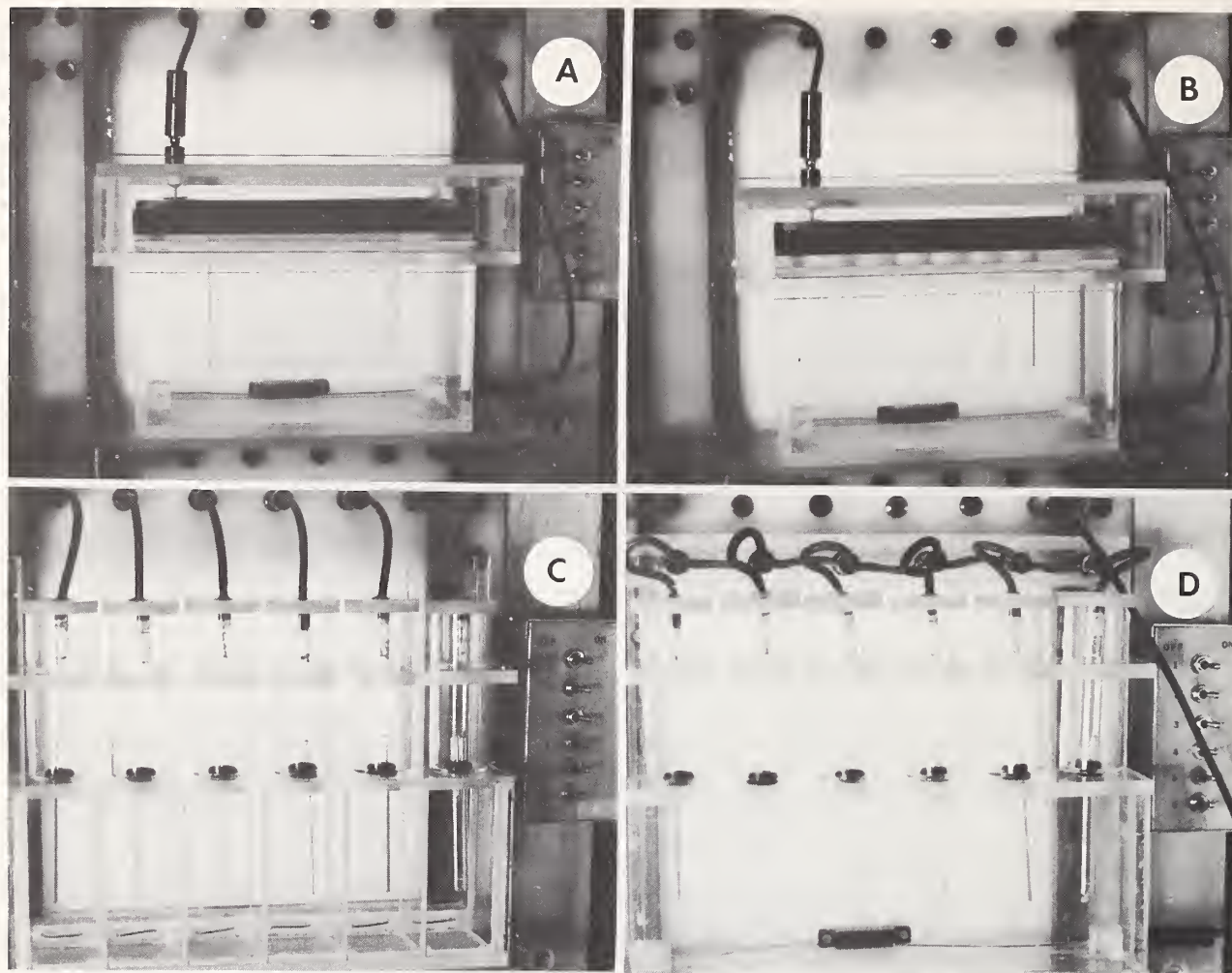


FIGURE 27.—Four apparatus that can be simultaneously run on the electrophoresis support station and controlled by the constant-current regulator. A, Vertical-slab gel; B, disk gels in vertical-slab-gel-bath apparatus; C, six independently controlled disk gels; D, disk-gel apparatus (after Besaw 1972).

ever, at higher currents and longer running times, larger cathode buffer reservoirs are advisable. These are easily fashioned (Besaw et al. 1972).

Construction of the disk-gel baths is straightforward and will not be dealt with in detail except for a few points that are not obvious from the figures. After the electrodes have been wired to the bottom baths as shown in figure 25, the troughs containing the wiring are filled with silicon rubber to imbed them permanently and to protect the operator from accidental contact.

The upper electrodes are fashioned to fit the lips of the plastic vials used as upper baths (fig. 24). A $\frac{3}{32}$ -in-wide circular groove that allows the electrode to snap in place and be tightly held by the lip of the vial is first routed one-eighth in into the $1\frac{3}{8}$ -in-o.d. acrylic ring. A $\frac{3}{16}$ -in hole is drilled

through the center of the disk. Over the top of this hole, drill another halfway through the disk, making the hole just wide enough to allow a 1-in piece of disposable plastic 5-ml pipet to seat firmly in place. Drill a number of $\frac{1}{8}$ -in holes along the 1-in piece of pipet to allow escape of the gases that are formed during electrophoresis. Form a loop at one end of the platinum electrode just large enough to pass through the lumen of the pipet, and bend it to a 90° angle with the shaft. Force the shaft end through a rubber septum, and then force the septum into one end of the pipet so that the platinum loop is flush with the other end of the pipet, but does not extend beyond the lumen. Solder a 4-in length of wire to the electrode protruding from the septum. Pass the wire through the hole in the disk, and

cement the pipet-electrode complex into place. A drop or two of silicon rubber is added to the septum surface within the pipet and to the hole of the outer ring surrounding the wire to seal it permanently in place. A jack of the appropriate color is attached to the free end of the wire (make half of the electrodes with red jacks, the other half with black jacks to correspond to the color code on the support station). Figure 26 shows a simple holding stand for the 72 upper bath electrodes, which keeps them from being damaged when not in use.

The rubber grommets used for holding the gel tubes in place on the upper bath reservoirs can be of various sizes, depending on the size of gel tubes used, but should be of a diameter slightly smaller than the outside diameter of the tubes employed to prevent leakage. The grommets can be cemented to the reservoirs with silicon rubber to prevent leakage from around their outer edges.

The design of the disk-gel equipment described can be easily modified to form an apparatus similar to that described by Besaw et al. (1972). The dividers of the lower bath are removed, and a platinum electrode extending the length of the bath, similar to that for the vertical-slab-gel apparatus, is substituted for the six individual electrodes. Six jacks connected in series are attached to the upright of the bath, and the same upper bath electrodes are used. With this arrangement, any of the six gels can be easily removed. When used in conjunction with the constant-current regulator, the disk-gel baths are connected to the circuit in the same manner as the vertical-slab-gel baths (fig. 27D).

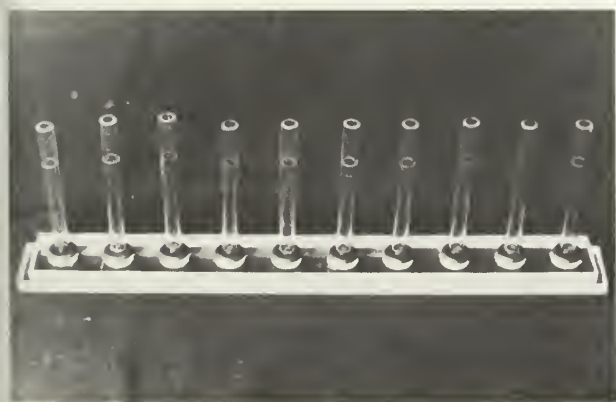


FIGURE 28.—Disk-gel-casting stand.

DISK-GEL-CASTING STANDS AND STORAGE BOX

Construction of the casting stands is simple (fig. 28). After holes of the appropriate size for the rubber grommets are drilled into the upper piece of acrylic plastic, it is cemented to the lower piece. An $\frac{1}{8}$ -in.-deep layer of self-leveling silicon is poured into each hole and allowed to cure. The grommets are then pressed into place. Use grommets that have a slightly smaller inside diameter than the size of the tubing to be used for gel casting. The use of different-sized grommets to fit the tubing allows various-sized casting stands to be fashioned.

When the columns are put in place, they must be perpendicular to the base so that the gels polymerize with a perfectly horizontal face.

If gels are to be stored for a day or two before use, they can be held at 5° C in a 12- by 6- by 4½-in.-i.d. acrylic plastic box. The gels are left in the casting stands, the water layer is removed, and the gels are placed in the storage box. A small amount of water is poured into the bottom of the box, and a piece of filter paper, soaked in water, is placed on top of the tubes. A saturated atmosphere is obtained, keeping the gels from drying out. For longer storage periods, the gels are removed from the casting stands and stored in sealed plastic bags that have 10 ml or so of gel buffer in them.

DISK-GEL-COLUMN MAKER

The disk-gel-column maker is a block of acrylic plastic with holes drilled into it to make gel columns of the desired size (fig. 29). The column maker described here was designed for making columns of either 75 mm or 100 mm total length out of 5-mm-i.d. by 7-mm-o.d. stock. Both tubes are permanently marked with a diamond pencil at 5 mm and 20 mm from the ends, giving an effective separating-gel running length of 50 and 75 mm, respectively.

For operation, the stock tubing is inserted through hole A until it butts against the stop block. The diamond pencil is inserted into hole B (made just large enough for free passage of the pencil, with no side-to-side movement), and a light hand pressure applied. The tubing is then twirled to make the permanent 20-mm marking. The diamond pencil is transferred to hole C for

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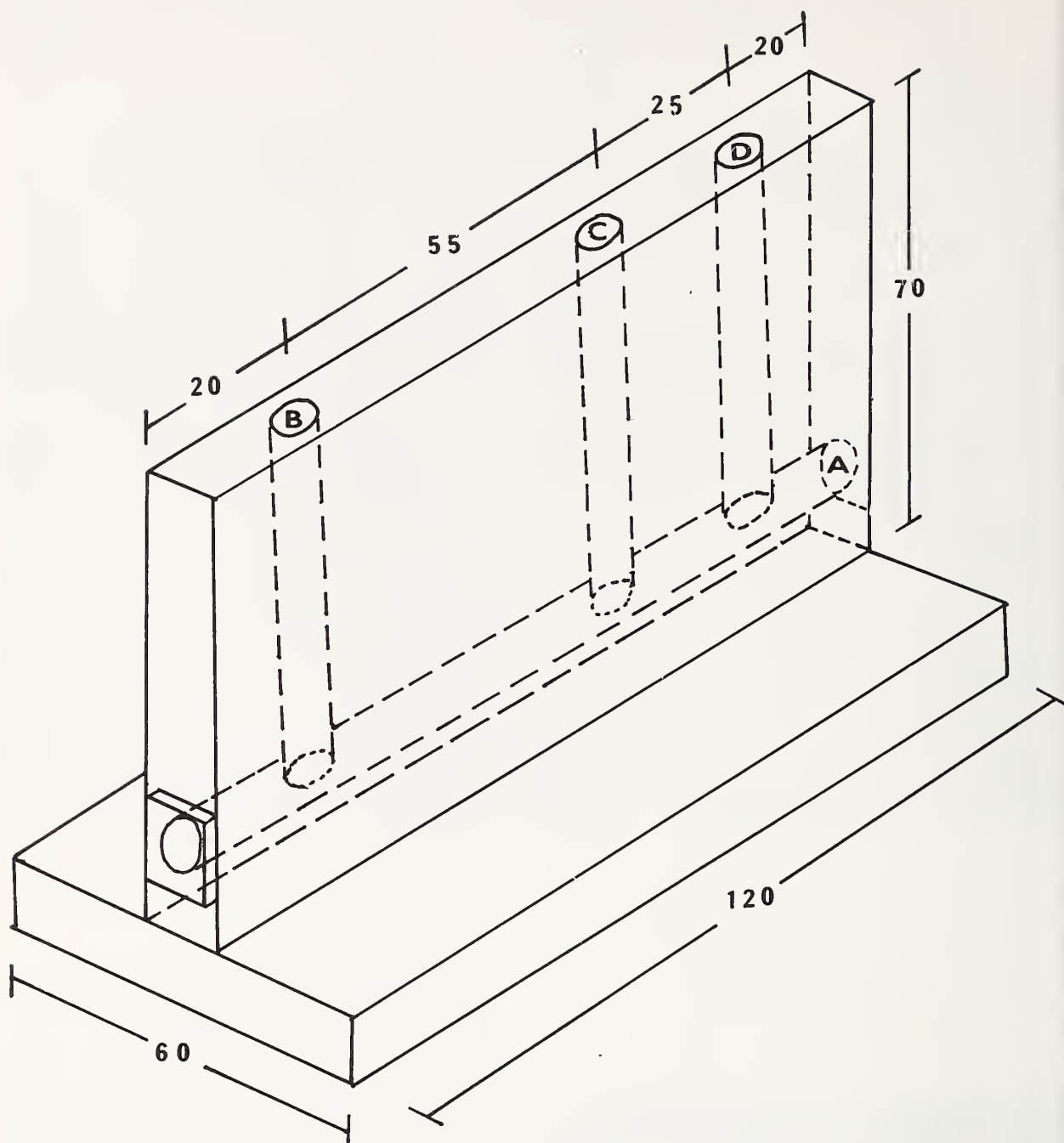


FIGURE 29.—Construction details of the disk-gel-column maker (measurement in millimeters).

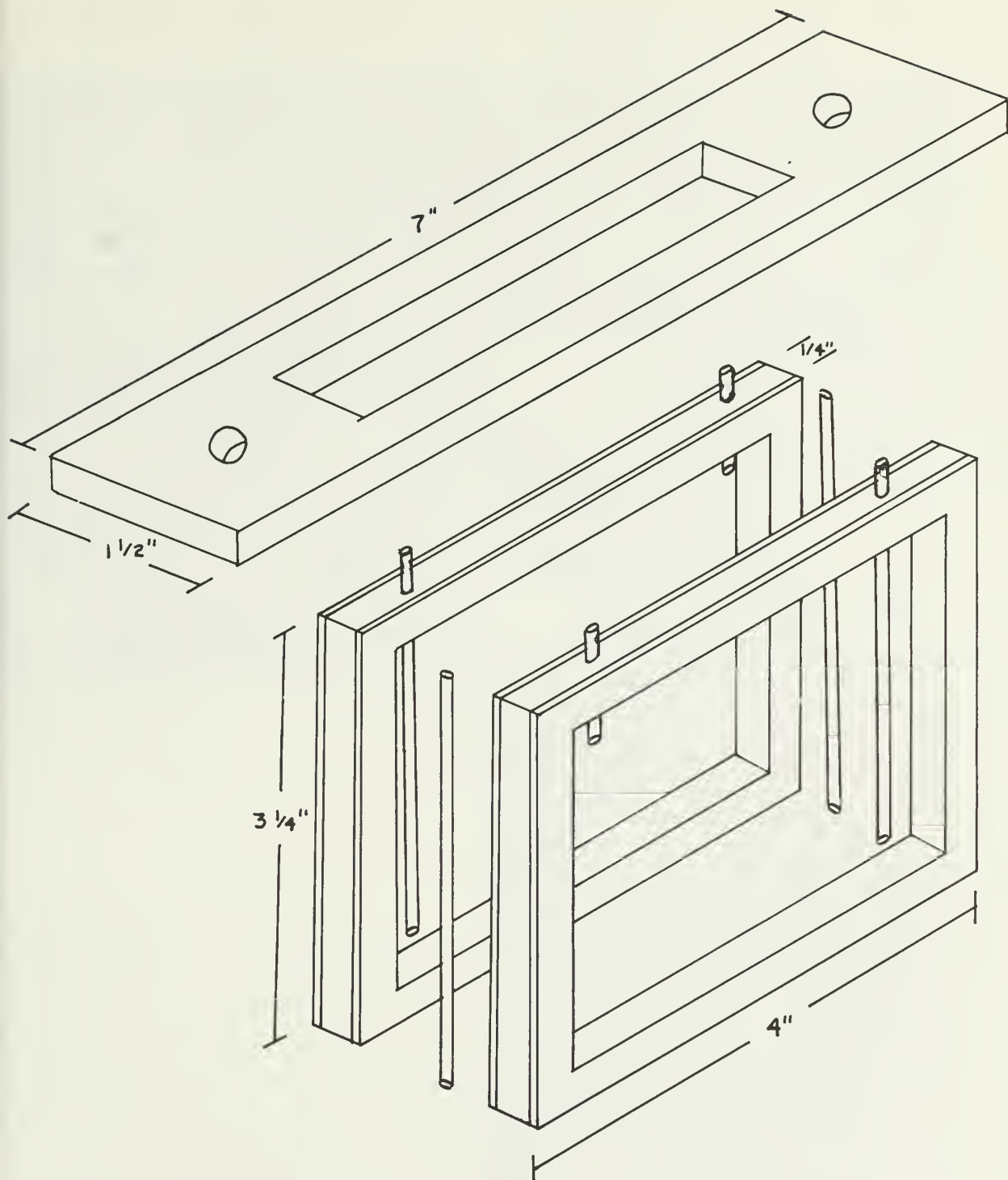


FIGURE 30.—Construction details of the vertical-slab-gel cooling-chamber cells.

75-mm columns or hole D for 100-mm columns, and a somewhat harder pressure applied while turning the stock tubing, resulting in a scratch completely around the tubing that makes breaking easy. After this mark has been made, the tubing is removed from the column maker, and the tubing is snapped by hand pressure with a thumb on either side of the mark. A 95-mm-long aluminum plug is inserted through hole A, and the end of the tubing opposite to the one containing the 20-mm mark is inserted until it is butted against the plug. The diamond pencil is inserted into hole D, and the 5-mm mark is made by light pressure on the pencil while twirling the column. The two cut ends of the columns are lightly polished with carborundum cloth, or on a carborundum wheel, to dull the edges. A disk-gel-column maker capable of making columns from tubing of any size and of any desired length and markings can be fashioned in this manner.

VERTICAL-SLAB-GEL COOLING-CHAMBER CELLS

Before the equipment of this system was mounted in a refrigerated cooler, it was used exclusively mounted on a cart. Because we were primarily interested in the study of insect isozymes, it was desirable to keep the sample and gels as cool as possible during an electrophoretic run to avoid denaturation of the enzymes. The coolant system described previously was developed, and specialized gel cells were constructed. Essentially, these were all-glass cells with a coolant chamber permanently attached to each of the glass faces (fig. 30). Although these cells are adequate, they were abandoned after the refrigerated cooler became available, mainly because of the reduction in steps made possible by utilizing buffer cooled at 5° C without a circulator.

With a jigsaw, the interior is cut out of a 4- by 3¼- by ¼-in piece of acrylic plate to form a frame with ¼-in sides. A ⅛-in hole is drilled through the top of these frames one-half in from either end, and 1-in lengths of ⅛-in-i.d. by ⅛-in-o.d. glass tubing are attached with epoxy cement to one hole in each frame, allowing a ½-in nipple to protrude from the top of the frame. To the remaining holes, a 3¼-in piece of glass tubing is likewise attached. With a thin layer of silicon rubber adhesive, a 4- by 3¼-in projector slide cover glass is attached to one face of the frames so that when the glass faces are in contact, one

short and one long glass tube are opposite one another. After the adhesive has cured, any adhesive that has oozed from between the contact points is cleared away, and a 4- by 3¼- by 0.02-in piece of acrylic plate is permanently attached to the open faces of the frames to form a watertight chamber.

To form the sides of the gel cells, disposable glass sample pipets are placed against the cell faces at either end, and tape is placed at the four open edges to hold the two cell chambers tightly together, as explained in the following section. Silicon adhesive is run down the outside edges of the junction of the pipets and the glass plates to seal them. The size of the gel chamber can be varied, up to about 3 mm, by using pipets or solid glass rods of different diameters. Before use, the lumens of the pipets are filled with molten wax to prevent buffer leakage.

To complete the cells, tops are made from acrylic strips measuring 7 by 1½ by ¼ in. A slot, just large enough for the cell complex to fit snugly, was centrally routed into it, and silicon adhesive was used to form a watertight seal.

In practice, after completion of an electrophoretic run, the chamber is forcibly separated from the top, and a razor blade is run along either edge between the glass pipets and the glass plate to free the two halves and to recover the gel slab intact. To form a new cell, reassemble the two halves after thorough cleaning.

DISPOSABLE VERTICAL- SLAB-GEL CELLS

The supplies required for the construction of the disposable glass cells are disposable glass microsampling pipets, 20-μl size, obtained from Corning (other sizes can be used, depending on preference), 3¼- by 4-in projector slide cover glass obtained from Kodak, RTV-112 white pourable silicon rubber adhesive obtained from General Electric, Scotch super-strength adhesive from 3M Company, Paraplast tissue embedding medium, and acrylic plastic.

The lumens of the pipets are first filled with molten Paraplast to prevent buffer leakage. Be sure to allow only the tip of the pipet that is to be cut away to be dipped into the wax. If the wax is present on that part of the pipet that will be used in the cell, polymerization will not occur at the acrylamide-wax interface.

With the holder illustrated in figure 31A, sand-

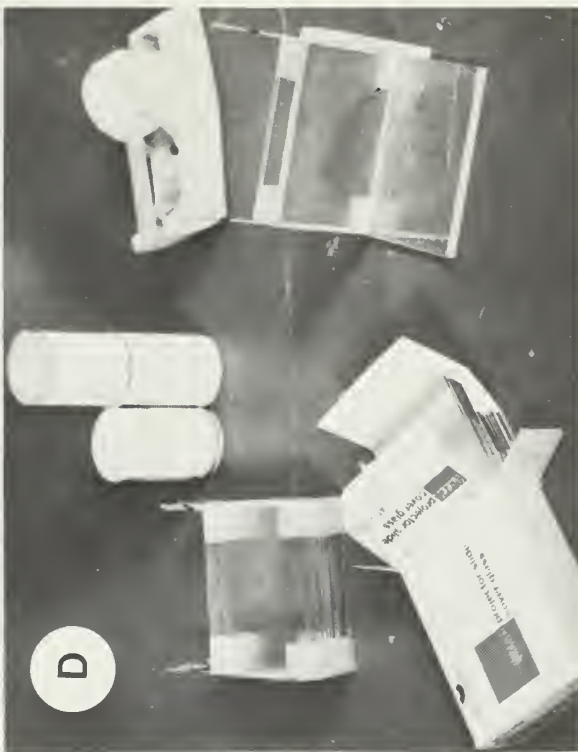
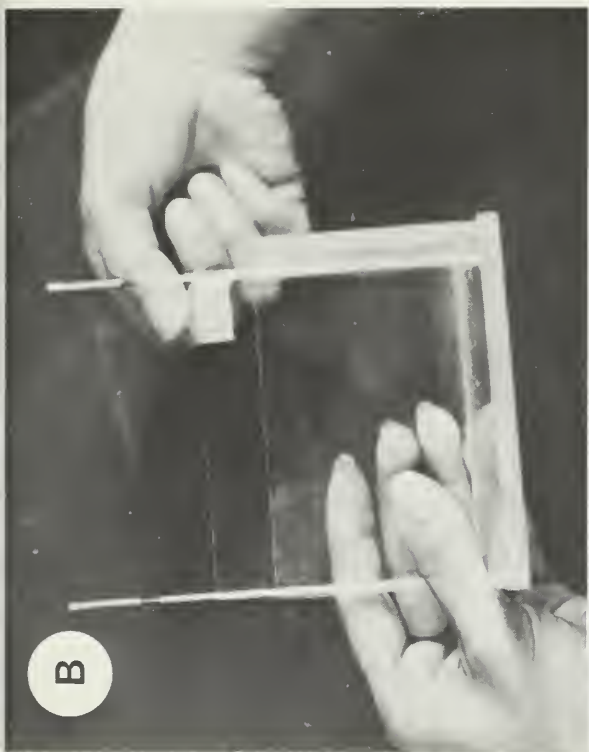
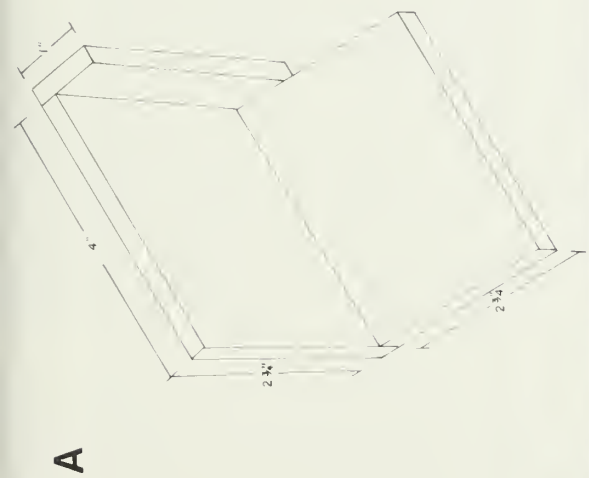


FIGURE 31.—Manufacture of the disposable vertical-slab-gel cells. A, Holder; B, taping of the glass sides with the aid of the holder; C, taping of the bottoms of the glass sides; D, stack of partially completed cells and the supplies needed for their construction.

wich two of the pipets between two glass plates, and place Scotch tape at the two upper corners across the open ends of the plates (fig. 31B); do not cut the pipets to size yet. Make sure that the pipets are flush with the plate sides and square with the bottoms (the primary purpose for the holder). Tape the glass plates together as tightly as possible. Holding the cell free of the holder (fig. 31C), tape the plates together tightly at the open ends of the bottom. If this has been done properly, you should not be able to move the pipets when a moderate pressure is exerted in trying to wiggle them from side to side. This is an important step, because the adhesive must not seep between the pipets and the glass plates.

After a number of cells have been fashioned in this manner (fig. 31D), place them onto the gluing frame (fig. 32), interspersed with 3- by 4-in sheets of $\frac{1}{8}$ -in thick polyethylene, making sure that all the cell ends are flush (fig. 32C). Tighten the sandwich of cells with the end plate so that they cannot slip. Place the complex on its side so that one side of the cells is uppermost. Apply Scotch super-strength adhesive onto the surface, and gently spread it over the entire exposed surface of the plate ends, being sure to cover completely the grooves formed by the pipets and the glass plate junctions (fig. 32D). The depth of the adhesive should not exceed about 1 mm. Let the cells stand undisturbed about 15 minutes. After the adhesive has set, repeat this procedure to seal the other side. The interiors of the cells should be completely devoid of adhesive. Because of the curved shape of the pipets, the acrylamide solution barely makes contact with the adhesive, assuring polymerization with no channeling.

At this point, with a carbide-tipped scribe, cut the excess length of pipet flush with the top of the cells. Remove the Scotch tape, and clean the cells with any good detergent with the aid of pipe cleaners. Use plenty of distilled water to wash all traces of detergent from the lumen of the cells. During preparation, avoid touching the side of the glass plates that will form the inner walls of the cells. Because the plates have been acid-washed during their manufacture, cleaning is much easier if fingerprint oils are not present. Store cells in a clean, dust-free atmosphere until ready to use.

To use the cells with the vertical-slab-gel baths, it is necessary to attach them to a Plexiglas support, a rectangular piece of acrylic plastic

with a groove routed into the middle that accommodates the glass cells (fig. 33, A, B). An aid used to attach the vertical-slab-gel cells to the top supports is shown in figure 34. The frame is made so that when the tops are in place, the cells will protrude through the slot one-fourth in, and be held perfectly vertical by the middle post (consisting of 1- by 3-in glass slides), which extends into the lumen of the cell. The tops are screwed down with the thumb-bolts to prevent any movement that could result in misalignment of the cells. A thin band of silicon adhesive, contained in a 10-ml disposable syringe, is applied completely around the cell-top interfaces to form a watertight seal and to hold the cells in a perpendicular position.

In this same manner, many of the commercially available preformed slab gels can be readily used with this apparatus. It is only necessary to fashion a top support with the appropriate-sized groove. Cells of different lengths or widths can be fashioned, depending on the size or configuration of the glass plates. A similar apparatus can be used for the assembly of the cooling-chamber cells.

When casting the gels, one of two ways is used to seal the bottom of the cell:

- (1) Use a water-resistant tape to seal the bottom, and dip the cell into molten wax to a depth of about one-fourth in above the tape; let the wax harden, and pour in the polymerization mixture. When the gel has polymerized, remove the tape and adhering wax with a razor blade.

- (2) Use an acrylic plastic stand with a groove that allows the cell to fit into it (fig. 33, A, E). Again, with silicon rubber adhesive, seal the bottom to the stand.

Plastic combs, with up to 24 equally spaced teeth (fig. 33, A, C), are used as sample slot formers. Their thickness should be slightly less than the lumen of the cells to prevent bulging of the glass sides, resulting in a separation between the gel and the glass and a channeling of buffer or leakage of sample between the gel and the glass.

Another item associated with these cells is a sample application guide (fig. 33, A, D). In practice, the glass cell is cemented to the top support with one-fourth in of the cell protruding from the top of the support, as mentioned earlier. After the sample slots have been formed and the cells are ready to receive sample (sample application

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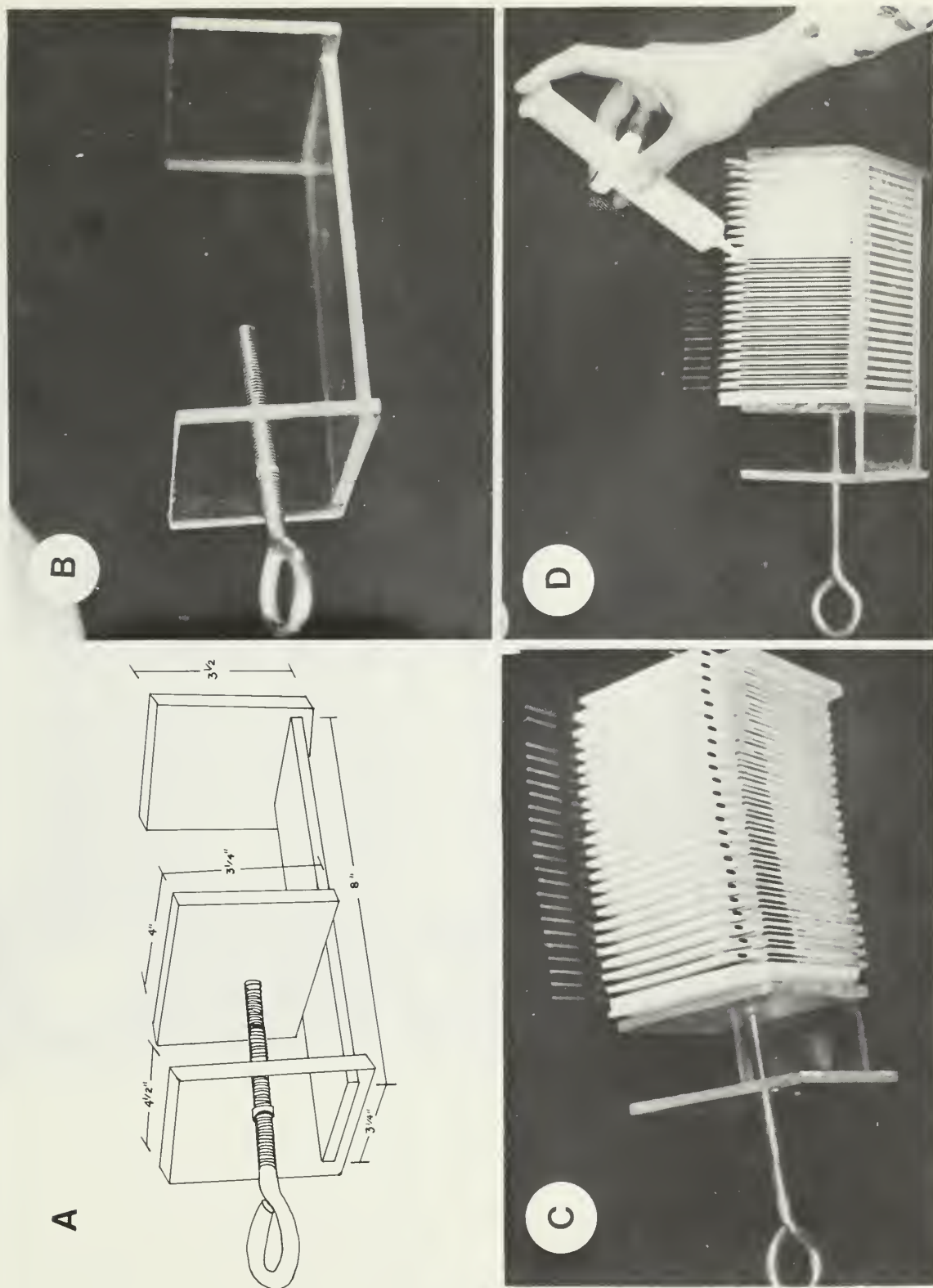
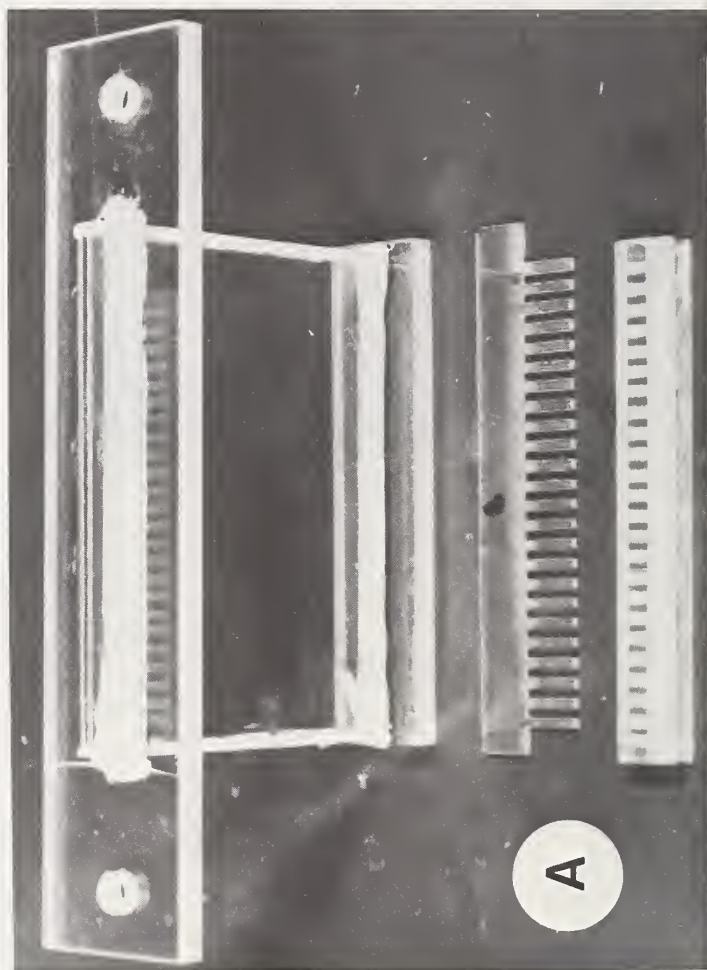


FIGURE 32.—Gluing frame. A, Construction details; B, gluing frame; C, gluing frame with disposable cells and polyethylene spacers; D, gluing frame on side and application of adhesive.



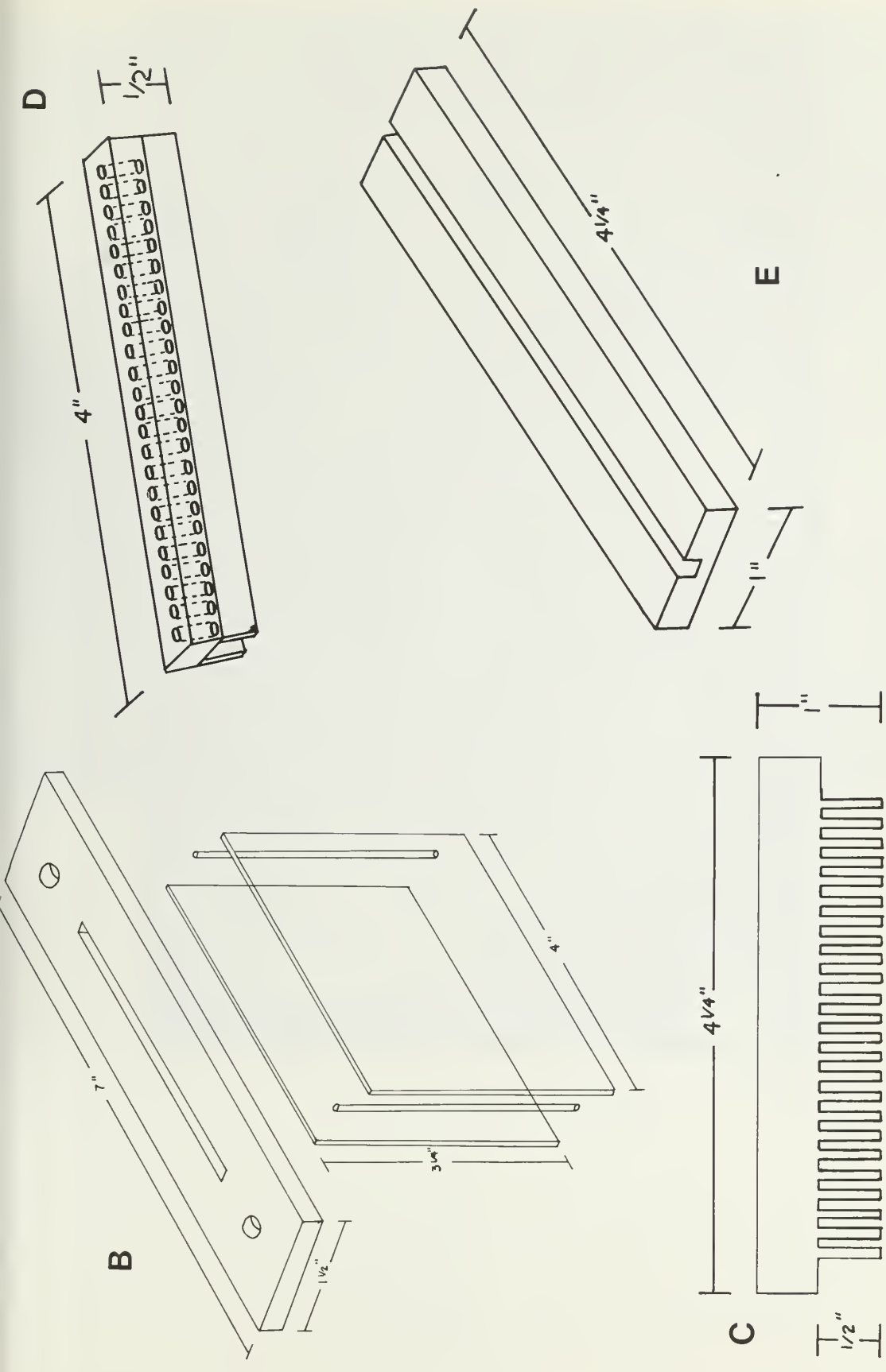


FIGURE 33.—Disposable vertical-slab-gel cells and associated apparatus. A, Completed cells and associated apparatus; B, construction details of the cell; C, 24-toothed, sample slot former; D, sample application guide; E, cell stand and bottom sealer.

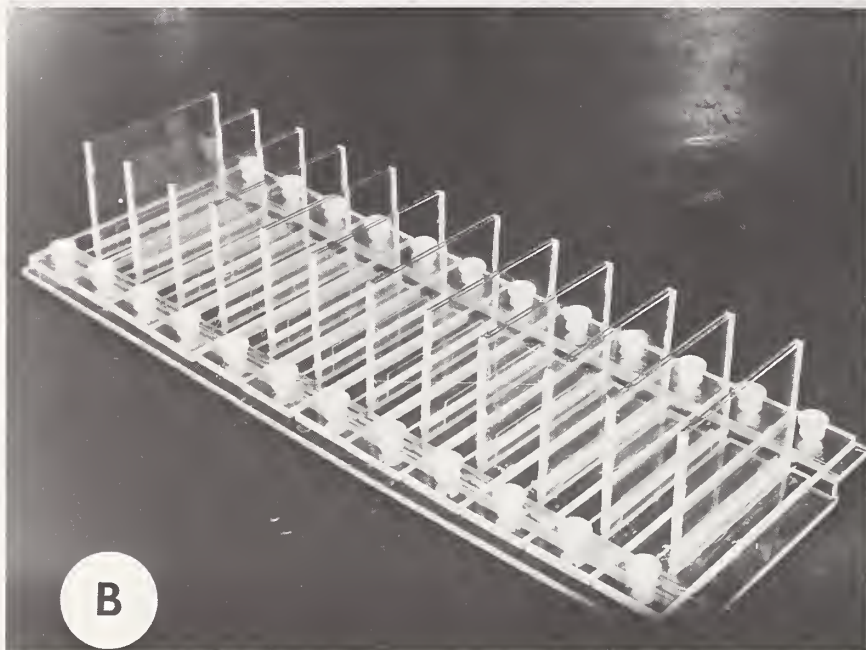
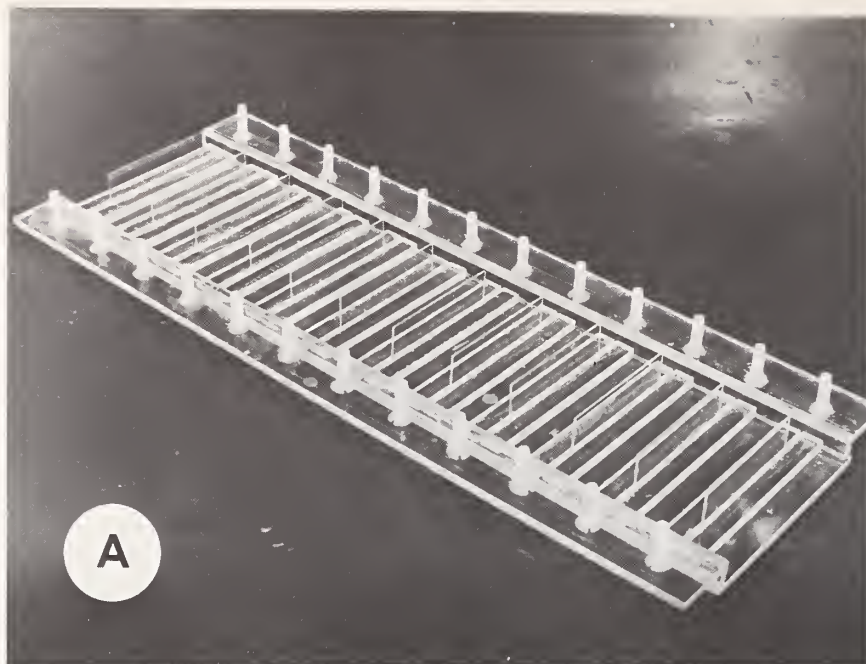


FIGURE 34.—Cell-top positioning frame. A, The frame, used to aline cells to a perpendicular position; B, a frame with 12 disposable cells positioned with tops.



FIGURE 35.—Constant-temperature polymerization bath (containing stock gel-forming solutions in black bottles, 10 vertical-slab gels and 2 disk-gel holders to be used in gel baths).

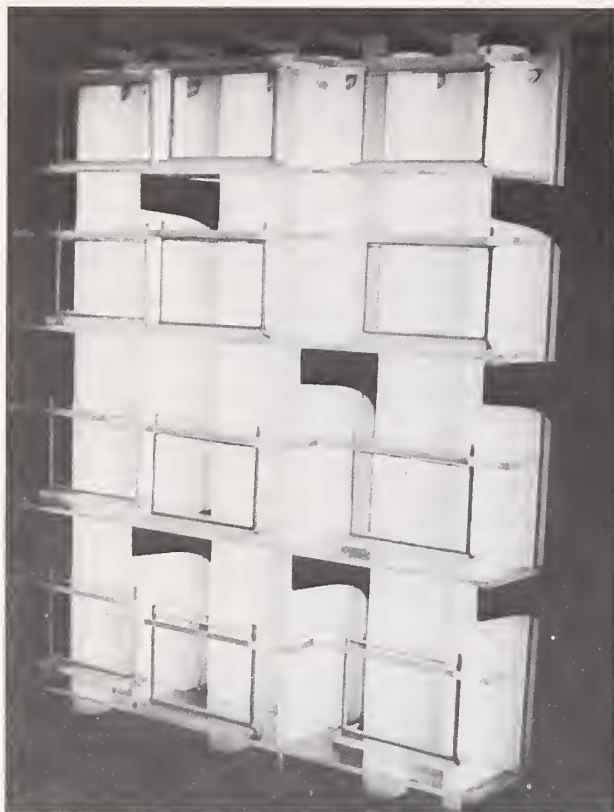


FIGURE 36.—Gel photopolymerization rack (with 12 vertical-slab gels).

is performed after the cells are mounted in the gel baths and buffer is present), the guide is placed over the cell by means of its flange, and the holes are lined up with the sample wells. A Hamilton syringe is used to apply the sample. Normally, the needle of the syringe is cut so that the base of the syringe rests on the top of the guide and the tip of the needle rests 1 to 2 mm above the sample-well bottom. A permanent mark placed between every fifth hole on the guide aids in applying the sample to the appropriate slot.

Disk gels can also be run in the vertical-slab-gel baths with a common upper and lower bath. Mount the gel columns in appropriate supports, as shown in figure 27D. Gel casting and polymerization can be done as explained in the next section.

CONSTANT-TEMPERATURE POLYMERIZATION BATH

Because the gel-polymerization rate is highly temperature dependent, it is necessary to control the temperature to achieve reproducible gels

(Maurer 1974). This control is satisfactorily accomplished by means of the gel bath pictured in figure 35. The procedure normally followed is to precondition the required amount of stock solutions contained in opaque bottles at the polymerization temperature. At the same time, 12 vertical-slab-gel cells with sealed bottoms are placed in the bath as pictured for preconditioning and, incidentally, to check for leaks. Gel-casting procedures, to be explained later, are carried out at a controlled temperature.

Disk gels may also be polymerized in this apparatus. Polymerization temperatures normally used are either 5° C or 25° C, with the temperature being controlled with either a refrigerated circulator or a heating circulator, as pictured in figure 35.

PHOTOPOLYMERIZATION RACK

The photopolymerization rack is of straightforward design as illustrated in figure 36. The shelving can be adjusted to support the different cell configurations. The rack is mounted on a platform that allows it to be tilted forward (after removal of the shelves and brackets) and on top of the constant-temperature photopolymerization rack. The light source is used in this fashion to photopolymerize the stacking gel layer in both the vertical-slab-gel and disk-gel configurations. It is not necessary to remove the gels from their constant-temperature environment until fully made. The lights consist of six 24-in, 20-W daylight fluorescent tubes with appropriate bases and ballasts.

METAL SUPPORTS

Ortec glass electrophoresis cells have been used extensively with the multifunctional electrophoresis system (McDonald et al. 1975; McDonald 1976; McDonald and Johnson 1976). Gel casting has normally been done with Ortec gel-casting stands, or similar stands of homemade design. A major problem was that cracks or breaks developed in the plastic tops at either end where the plastic contacts the glass. This problem was caused by the slight bowing of the plastic at this junction, when the wingnuts were tightened to prevent bottom leaking. To remedy this situation, metal supports (fig. 37A) are employed between the plastic tops and the wingnuts, as illustrated in figure 37C. The metal supports

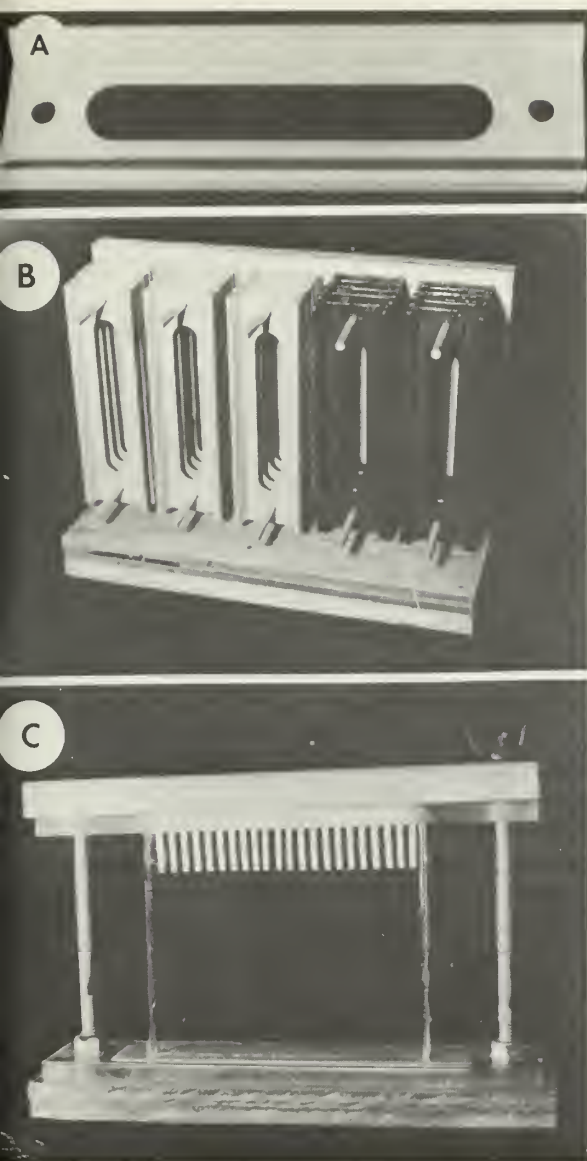


FIGURE 37.—Metal supports. A, Epoxy-coated metal support; B, metal supports on a storage rack; C, metal support in use on a homemade gel-casting stand.

cause an even pressure over the entire top, preventing stress.

The metal supports also have proved useful in the vertical-slab-gel baths when the Nalgene gaskets are used (fig. 27, A–D). Without the metal supports, the plastic cell tops have a tendency to bow slightly when clamped down with the nylon thumb-bolts used to seal the bath and the cell by means of the gasket. The bowing can lead to leaks resulting from uneven pressure over the length of the gasket. The metal supports completely eliminate this problem. In order to pre-



FIGURE 38.—Enzyme-reaction chamber showing previously developed gel and syringe method of filling.

vent rusting or electrolysis of the metal, the entire support is coated with epoxy paint, and to prevent chipping, the supports are stored upright (fig. 37B).

ENZYME-REACTION CHAMBERS

One of our major concerns is the study of the electrophoretic enzyme polymorphisms of the boll weevil throughout its range. We routinely look at 24 different enzyme systems from each individual weevil. It has become necessary to utilize economically the various chemical constituents needed for the formulation of the specific enzyme-staining systems. A procedure is used to process each gel with only 10 ml of stain solution.

After electrophoresis is completed, the gel is removed from the cell and if necessary, placed in cold buffer for prestain treatment. If preconditioning is not necessary, the gel slab is placed onto a 4- by 5-in sheet of $\frac{1}{8}$ -in-thick plate glass. A $\frac{1}{4}$ -in-thick polyethylene gasket measuring 4 by 5 in o.d. and $3\frac{1}{4}$ by $4\frac{1}{4}$ in i.d. is placed on

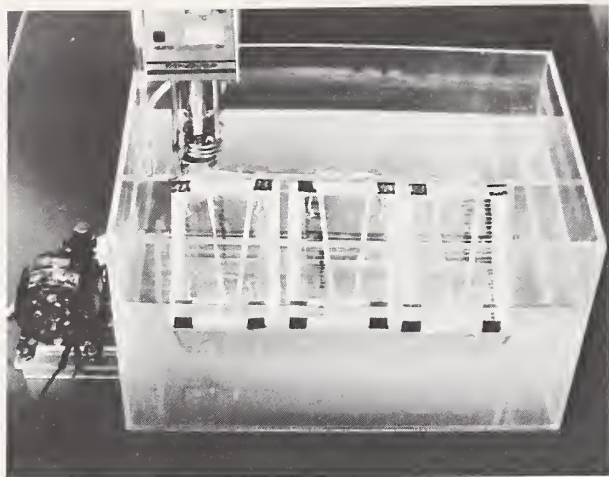


FIGURE 39.—Rotary temperature bath showing three gels attached to one of four faces of the square, rotating drum.

top of the glass plate with the gel in the middle. A second piece of 4- by 5-in plate glass is placed on top, and the sandwich is clamped together with four to six small paper binder clips and stored in a refrigerator until all gels (normally 12) are ready to be developed.

The appropriate substrate and staining solution, made in 10-ml quantities, are then injected by means of a 10-ml syringe and 22-gage needle through the gasket at one corner (fig. 38), and the reaction chamber is placed on a rotary temperature bath for development.

ROTARY TEMPERATURE BATH

Once the gels contained in the reaction chamber are charged with the appropriate staining mixture, it is necessary to develop them at a proper temperature for enzyme visualization. This development is accomplished by placing the enzyme-reaction chambers onto a square, rotating drum contained in a water bath kept at a constant, predetermined temperature (usually 30° or 37° C). As shown in figure 39, the chambers are kept in place on the drum face with heavy-duty rubber bands. The rubber bands are held to the face by 3 pegs, 2 on top and 1 at the bottom of the drum face at each of the 12 chamber positions (3 on each of the 4 drum sides). The drum rotates at the rate of 17 r/min by means of an externally mounted 1/12-hp step-down motor attached to the drum shaft.

The gel is thus constantly bathed in fresh

staining solution. The slow rate of revolution of the drum prevents vigorous movement of the gel within the chamber, preventing gel breakage from being a problem. The thickness and size of the chamber is such that the gel cannot fold on itself, but is held more or less flat against the glass, and allows for any gel swelling that may occur.

In practice, the staining solutions, contained in black painted vials, are preconditioned in the water bath before injection into the chamber so that the gels can quickly be brought to the developing temperature. Although not shown in the photograph, the outside walls of the rotating temperature bath are painted black, as is a removable top. The black paint protects the components of the staining solution from light, because many of them are light sensitive. This precaution results in gels with much clearer backgrounds than would be possible if the reactions were carried out in room light.

The progress of gel staining is observed by periodically lifting the lid of the bath and observing the gels. After the gels have developed to the desired stage, they are easily removed while the drum is rotating by stretching the rubber band off the pegs. The chambers are then opened, and the gels are washed with running water. They are then ready for posttreatment processing.

GEL MEASURING AND DRAWING DEVICE

The gel measuring and drawing device is basically a caliper rigidly mounted on a Plexiglas frame, as pictured in figure 40. It was designed so that the distance traveled by proteins of interest could be measured from the gels themselves, or from photographs of the gels. Both disk gels and vertical-slab gels can be utilized in this device.

A Plexiglas strip is attached to the sliding jaw of the caliper. It has a thin line (cut with a razor blade) extending its length on both the upper and lower surfaces, which are directly in line with each other. These scribed lines are darkened with India ink so that they stand out boldly against the background. The purpose of the two lines separated by the 1/4-in thickness of Plexiglas is for parallax correction. As the eyes are moved over the top of the lines, both the upper and lower lines can be seen. At the point at which only one line is seen (when they are superim-

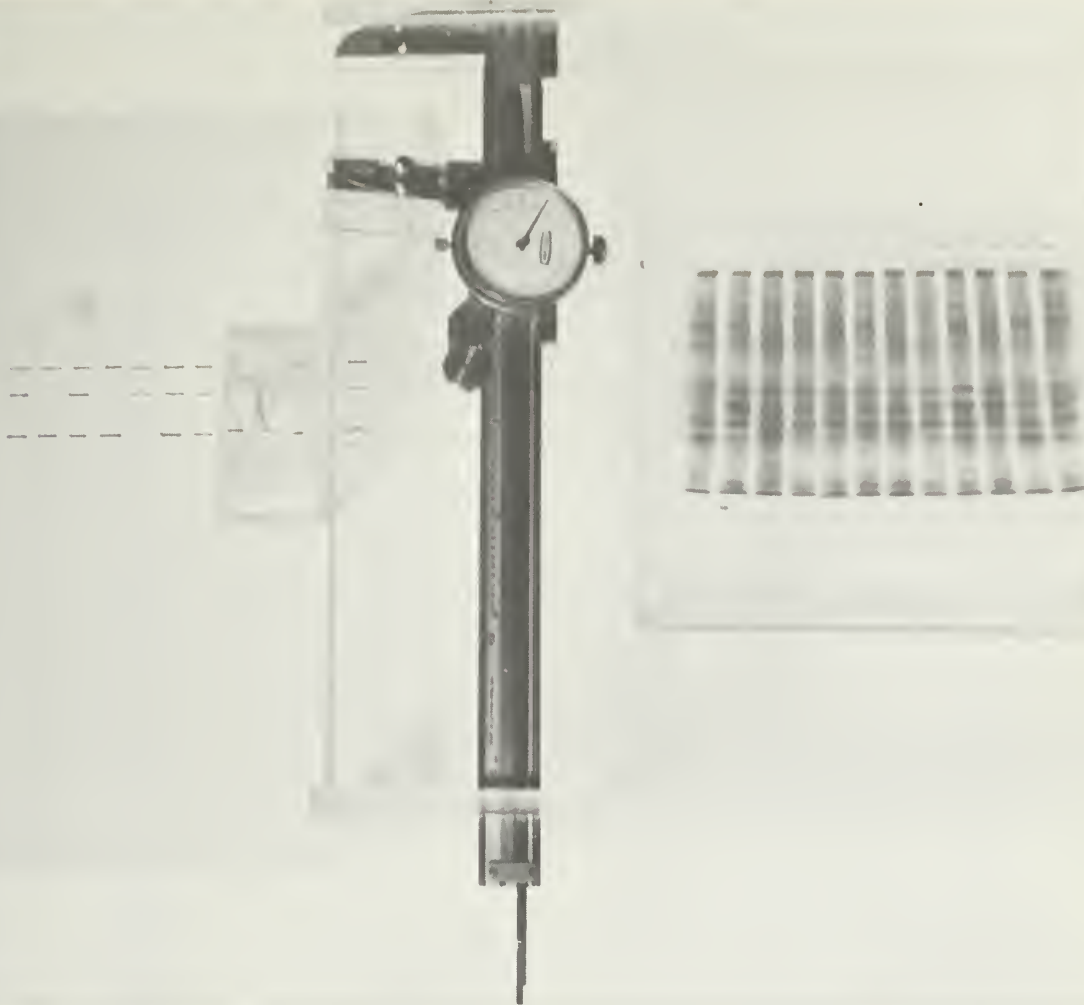


FIGURE 40.—Gel measuring and drawing device.

posed), reproducible measurements are possible, because alinement to the same spot is assured. A sliding pen holder is also attached to the sliding arm. With it, an accurate reproduction of the gel is possible.

As pictured, the gel is held in a transparent box free to move either up or down, or from side to side on rails above the strip bearing the scribed lines. The origin of the gel can thus be alined to the 0 point of the caliper. Photographs are similarly mounted beneath the strip.

Measurements are made by moving the caliper arm until the parallax-correcting lines are superimposed on the band of interest. A reading of the travel distance is recorded, and at the same time a drawing of the gel is made by sliding the pen to one side, as shown in figure 40. This procedure is repeated until all bands of interest have been

measured. A final measurement of the tracking dye front is made at each sample location, and the mobilities of the bands relative to the front are calculated.

COSTS

The prototype of this complex took 1 year to complete, including the time to order and receive raw materials, build and test the components, and change design and rebuild those components that did not perform satisfactorily. The cost of materials, excluding the commercially available power supplies, refrigerated cooler, and temperature controllers, was approximately \$1,300 (1974 prices). A second setup that includes all of the equipment mentioned in this report, again excluding the power supplies and so forth, took

Table 8.—Construction time and cost estimate for building the multifunctional electrophoresis system

Components	Time (hours) ¹	Cost (dollars) ²
ELECTRICAL AND COOLING NETWORK		
Constant-current regulator, option 1	120	520.00
Constant-current regulator, option 2	120	400.00
Alternating-current power-control safety-interlock system	2	20.00
Electrophoresis support station (with 3 separate circuits and coolant-distribution lines)	40	250.00
Cart	40	200.00
Coolant-valving station	16	35.00
Constant-flow manifold	2	10.00
Return manifold	1	5.00
Double-walled coolant box	10	50.00
ANCILLARY ELECTROPHORESIS EQUIPMENT		
Vertical-slab-gel baths, including		
platinum wire electrodes (12)	24	225.00
Disk-gel baths, including platinum wire electrodes (12)	24	225.00
Disk-gel-casting stands (12) and storage box	4	10.00
Disk-gel-column maker	1	1.00
Vertical-slab-gel cooling-chamber cells (12)	12	24.00
Disposable vertical-slab-gel cells (120)	8	36.00
Holder5	.50
Gluing frame	1	2.50
Acrylic cell tops (120)	16	12.00
Cell-top positioning frame	2	3.00
Cell-bottom sealing stand (120)	16	5.00
Sample combs (120)	8	6.00
Sample application guide (120)	8	5.00
Constant-temperature polymerization bath	4	10.00
Photopolymerization rack	8	30.00
Metal supports	8	12.00
Enzyme-reaction chambers (12)5	6.00
Rotary temperature bath	4	20.00
Gel measuring and drawing device	4	25.00
Total (option 1)	384.0	1,748.00

¹ Based on materials readily at hand and shopworking facilities available.

² Based on prices effective Sept. 1977.

about 3 months to build at a cost of approximately \$2,000 (1977 prices). A breakdown of the cost of materials and the time to build each component is presented in table 8.

PROCEDURES

Because so many noncommercial pieces of equipment form an integral part of the multifunctional electrophoresis system, a step-by-step approach is desirable to demonstrate their functions and interrelationships. First, preparatory steps leading to electrophoresis of vertical-slab and disk gels will be presented; second, electro-

phoresis parameters will be presented; and lastly, postelectrophoresis steps will be presented. These steps are typical of those used routinely in this laboratory on a day-to-day basis.

CHEMICALS

Electrophoresis-grade acrylamide, bis (*N,N'*-methylene-bis-acrylamide), temed (*N,N,N',N'*-tetramethylethylenediamine), riboflavin, ammonium persulfate, Coomassie blue R-250, and bromophenol blue were obtained from Bio-Rad Laboratories. All other chemicals were reagent grade from various manufacturers.

The gel system used to demonstrate the multi-

functional electrophoresis system was essentially that of Davis (1964), except that stock solutions C and D were modified to produce a 6.5 percent separating gel and a low bis stacking gel (McDonald et al. 1975).

Samples consisted of Electrophoresis Control from Gelman Instrument Co. and human albumin, myoglobin, and fibrinogen from Sigma, all diluted with sample buffer (upper gel buffer made to 20 percent sucrose) to a concentration of 2 μg per μl .

PREPARATION OF VERTICAL-SLAB GELS

Vertical-slab gel cells are normally constructed in groups of 120, complete with bottom supports. When ready to use, 12 cells are placed into the temperature-controlled photopolymerization rack, kept at $25^{\circ}\pm 1^{\circ}$ C with the individual gel components. These components are left to equilibrate for at least 1 hour. The cells are checked for leakage. If no leakage is observed, gel solutions A and C are mixed and de-aerated with a hand vacuum pump, as is solution G. These gel solutions are then combined carefully to prevent the introduction of air bubbles, and about 10 ml is pipetted into each cell to a depth of 65 mm. Each cell is immediately water-layered by gravity flow by means of a 1-ml disposable syringe equipped with a 26-gage, $\frac{1}{2}$ -in needle. The cells are left undisturbed for 1 hour for polymerization to occur (a thin, refractile line should be apparent at the end of 30 minutes, denoting that polymerization has occurred). After the 1 hour, the unpolymerized liquid is removed from the cells by tipping the cell to one side and sucking the fluid away with a syringe. The gel tops are then rinsed two times with the rinse solution.

The room is darkened, and the components for the stacking-well forming gel are mixed and de-aerated. About 2 ml of this solution is pipetted on top of the separating gel, and the sample-well forming comb is put into place to the shoulder. This process results in a 5-mm layer of stacking gel as measured from the bottom of the sample well to the top of the separating gel. Either the gels are placed about 5 cm in front of the polymerizing light source, or the light source is lowered on top of the temperature-controlled polymerization rack, whichever is preferred. Photopolymerization is allowed to occur undisturbed for 1 hour (30 minutes is necessary for

polymerization). One at a time, the bottom support is removed from each gel cell by first rimming the silicon adhesive with a single-edge razor and carefully pulling the plastic support free. A smooth-bottom gel surface flush with the glass sides should be seen. If gel tabs are present, these can be removed with the razor.

The cell is submerged into distilled water and the comb is carefully pulled free. A squire bottle containing upper buffer is used to displace the water and unpolymerized gel in the sample wells.

The vertical-slab gels are mounted into the upper buffer baths and placed into position on the prefilled lower buffer baths. After all 12 gels are in place, pour about 300 ml of upper buffer into their baths, and dislodge any air bubbles that may be trapped in the sample wells with a syringe containing upper buffer. The sample guides can be put into place at this point if they are to be used.

PREPARATION OF DISK GELS

Preparation of the disk gels is essentially identical to the method of Davis (1964). If a full 72-tube run is contemplated, however, prepare the separating gel in small portions (enough for about 12 gels) to avoid the possibility of the gel mixture polymerizing before all tubes are filled and water-layered. A rule of thumb used in this laboratory (for both disk and vertical-slab gels) is to mix only enough gel solution to complete the steps through water-layering in 5 minutes.

SAMPLE APPLICATION

When the sample guide blocks are used, a Hamilton syringe is normally used to apply the samples. The syringe needle is cut so that the glass shoulder of the syringe rests on top of the guide block and the tip of the needles lies 1 to 2 mm from the sample-well bottom. From 10 to 20 μl of sample volume containing 20 percent sucrose is applied to each slot. If the sample guide is not used, any pipet or syringe can be used, as long as the tip is small enough to fit into the well. A 10 or 20 μl Eppendorf pipet is ideal for sample application, as is a Hamilton 250 μl needleless syringe fitted with a repeating adapter and an Eppendorf yellow pipet tip.

If the bromophenol tracking dye does not interfere with the sample, an amount sufficient to give a bluish tint to the sample solution can be

added as an aid in finding the sample wells. With practice, the slight difference in refractive index between the gel and the wells becomes evident, and the dye is not necessary.

Once the samples are put on, the baths are carefully transferred to the electrophoresis support station and electrically connected to the proper terminals.

ELECTROPHORESIS

(1) *Electrophoresis of 12 vertical-slab gels with the constant-current regulator and 1 power supply.*—Connect the cathode (upper bath) to jack J11 of the support station at each of the 12 positions. Likewise, attach the anode (lower gel bath) to jack J16. Turn switches S11–15 off and switch S16 on at each of the 12 positions. Turn the master power switch S8 on, and check the operation of the safety-interlock switch by partially opening and closing both cooler doors. A clear click denotes that this circuit is working properly. Place the power supply to the constant-voltage position, and attach the three terminal power leads to the first pair of jacks on the face of the power supply board directly beneath three of the high-voltage power supplies (fig. 5). Place the power supply to the operating position, and adjust it to 400 V. Place the 12 range switches (S5) of the constant-current regulator to 10X, and turn on the power. Consecutively, place the 12-position select switch (S7) to positions 1 through 12, and place the normal-off-reverse switches (S6) to the normal position, while adjusting the current to the desired milliamperage reading (up to 25 mA/gel) at each position.

At the end of the run, back off the current adjust knob for that cell to 0 mA, place switch S6 to the off position, open the cooler door (activating the safety interlock), turn switch S16 to the off position, and remove the gel bath. Proceed in this fashion until all 12 gels are removed or turned off. When gel number 12 is turned off, turn off the constant-current regulator, the power supply, and the master power switch.

(2) *Electrophoresis of 12 vertical-slab gels with the constant-current regulator and 3 power supplies.*—The operation is identical to the previous section except that four gels are powered by each of three power supplies. Each power supply has to be turned on and independently adjusted to 400 V. Each power supply should be turned off when the last of the four gels con-

trolled by it is finished electrophoresing. Also, the power leads are attached to only the first set of jacks directly beneath the power supply. With this arrangement, a maximum of 35 mA per gel is possible.

(3) *Electrophoresis of four vertical-slab gels with constant current or voltage with one power supply by means of the auxiliary circuitry.*—With this arrangement, the constant-current regulator is completely bypassed, and the four gel stations are controlled directly with the power supply. The upper baths are electrically connected to jacks J18–21, respectively, and the lower baths to jacks J22–25, respectively, for anodal runs. If cathodal runs are desired, reverse this procedure. Switches S17–20, contained within the cooler, are switched to the on position. The power supply is electrically connected to this circuitry by means of the second set of jacks located directly below it. The power supply is set to the desired electrophoretic conditions with its controls.

A maximum of 25 mA/gel or 250 V/gel is possible with all four gels operating; with less than four gels, these values are proportionally higher. When each gel has been electrophoresed to a desired point, its control switch is turned off, and the gel is removed from the system. The power-supply voltage or amperage, whichever the case, will drop accordingly, but will have no effect on the settings of the remaining gels.

(4) *Electrophoresis of two vertical-slab gels with constant power with one power supply.*—Constant-power runs are made with the cells connected directly to the power supply by means of the direct circuitry provided (the last three groups of jacks located directly below the power supplies on the face of the panel). Two vertical-slab gels per power supply can be powered in this manner by means of the two sets of jacks on the power supply.

(5) *Electrophoresis of 24 disk gels, individually, with the constant-current regulator and 1 power supply.*—Connect the lower bath to the six-contact strip (J17) located on the translucent face of the support station by means of the six-contact edge board on the back of the bath. Connect each of the upper electrodes to jacks J11–16, respectively, and place switches S11–16 in the on position. Turn master switch S8 on, and check the safety-interlock circuitry as before. Place the range-selector switches of the constant-current regulator to the 1X position, and proceed



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FIGURE 41.—Polyacrylamide-gel fractionation patterns of human serum control run simultaneously on 12 vertical-slab gels (current = 35 mA/gel; load = 10 μ l/channel).

as in section 1, except place the normal-off-reverse switches in the reverse position for anodal runs.

The voltage of the power supply is adjusted to 875 to 900 V, and the current is adjusted to a maximum of 3.5 mA by means of the constant-current regulator. As each run is ended for a particular disk gel, its control switch (J11-16) is turned off, bypassing that cell. The gel can be removed from the apparatus for further treatment. Current to the remaining gels is not affected. If the individual gels develop more than a 150-V drop, the current will show a corresponding drop. With each buffer and gel combination, the maximum voltage through a run must be made empirically, and future runs must be powered accordingly.

(6) *Electrophoresis of six disk gels with individual upper baths and a common lower bath run with the constant-current regulator and one power supply.*—A total of 24 disk gels (4 groups of 6) can be run in this fashion. The individual upper electrodes are connected to the jack strip (series connected) on the lower bath frame (fig. 27D), and the common connection made to jack J11. The common lower bath is connected to jack J16. From here, the procedure is the same as outlined for section 2. Up to 35 mA (5.8 mA/gel) can be applied to each of the four groups of gels. Each gel can be individually removed from the apparatus. The current has to be adjusted downward to maintain a constant amount to the remaining gels.

(7) *Electrophoresis of 6 to 12 disk gels per station with common upper and lower baths.*—The procedure is identical to section 6 except that only two electrodes are involved: the common upper electrode and the common lower electrode. Most commercial forms of this technique can be utilized.

POSTELECTROPHORESIS STEPS

After electrophoresis, the vertical-slab gels are removed from the upper gel baths, and the plastic support is forcibly removed from the gel cell (the silicon adhesive used to hold the tops in place does not form a strong bond with the plastic). A razor blade run between one of the glass plates and the pipet end pieces separates the cell, leaving the gel attached to the other glass plate. The gel is carefully peeled from its support and placed in the appropriate solution for further treatment.

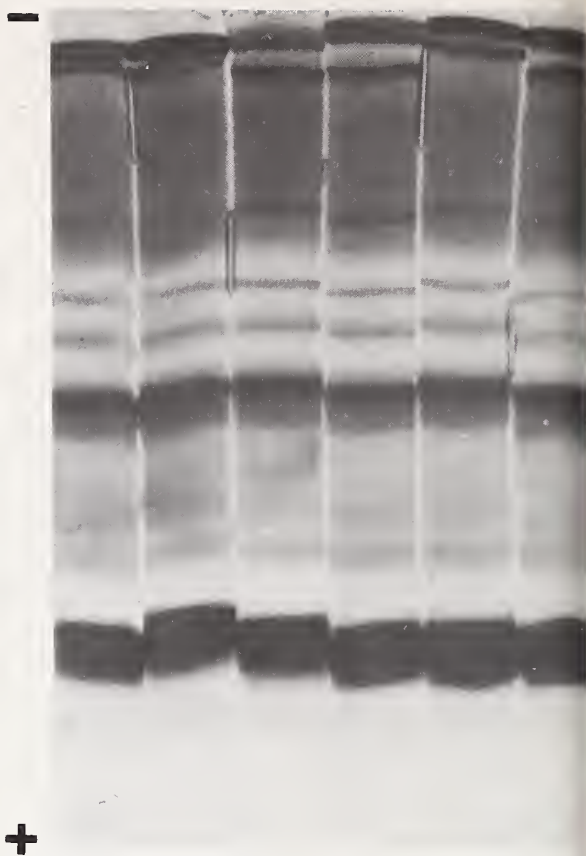


FIGURE 42.—Polyacrylamide-gel fractionation patterns of human serum control run by the disk-gel technique with the separate upper and lower bath apparatus (current=3 mA/gel; load=20 μ l/gel).

For the examples presented in this report, the gels were placed in 12.5 percent trichloroacetic acid for at least 30 minutes to fix the proteins. They were then stained overnight in 50 ml of 0.05 percent Coomassie Blue dissolved in 7 percent acetic acid, contained in Tupperware sandwich boxes. The excess stain in the gels was removed with repeated 50-ml portions of 7 percent acetic acid until the backgrounds were colorless.

The gels were transferred to gel reaction chambers fitted with a $\frac{1}{8}$ -in polyethylene gasket, as described earlier. The entire lumen of this sandwich was filled with 7 percent acetic acid by means of a syringe with special care to exclude all air bubbles.

The gels were either photographed on 35-mm Panatomic X film with transmitted light, or placed directly on photographic paper, and a contact print was made.

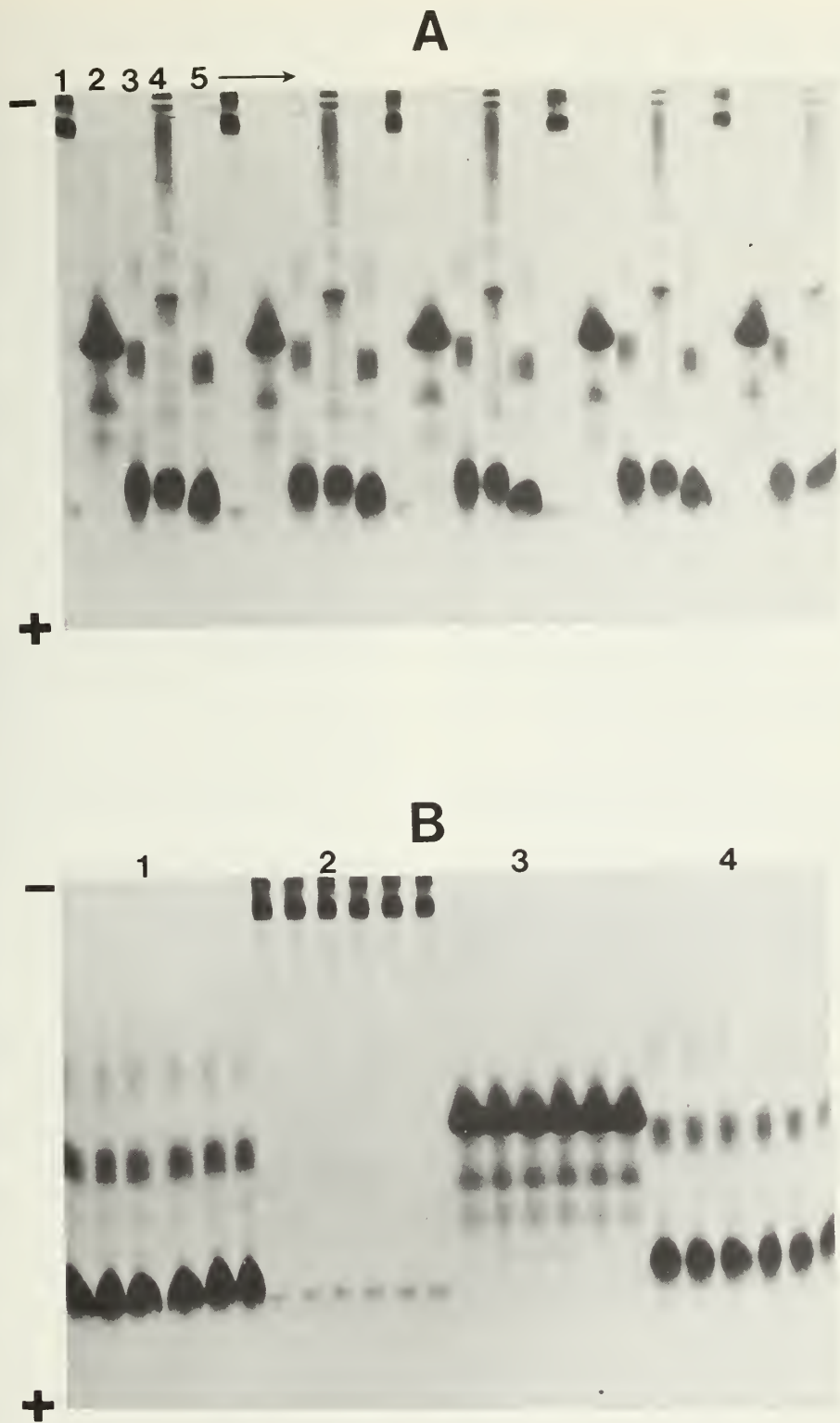


FIGURE 43.—Polyacrylamide-gel fractionation of various protein standards. A1, Fibrinogen; A2, myoglobin; A3, human albumin; A4, human serum control; A5, bovine albumin; all samples run on a vertical-slab gel with the optional circuitry discussed in the text (current = 30 mA/gel; load = 10 μ l/channel). B1, Bovine albumin; B2, fibrinogen; B3, myoglobin; B4, human albumin on vertical-slab gel; all samples run directly from the high-voltage power supply (current = 30 mA/gel; load = 10 μ l/channel).

The gels were placed on the gel measuring device (described earlier), and measurements were made of the distance traveled by the tracking dye and of the proteins of interest for each sample. The gel values were calculated according to the method outlined by Rodbard and Chrambach (1974). The gels were then sealed in polyethylene bags for permanent storage.

PERFORMANCE

To demonstrate the overall performance of the multifunctional electrophoresis system, some representative photographs depicting the results obtained with this equipment are shown.

Figure 41 shows a fractionation of human serum control on 12 vertical-slab gels run simultaneously with the constant-current regulator under the conditions reported in the text.

Figure 42 shows a fractionation of human serum control on six disk gels run simultaneously and independently with the constant-current regulator and the bath apparatus shown in figure 24.

Figure 43A shows a fractionation of various proteins run with the vertical-slab-gel bath and the optional circuitry shown in figure 11, and figure 43B shows a fractionation directly controlled by the high-voltage power supply. Other applications have been previously reported (McDonald et al. 1975; McDonald 1976; McDonald and Johnson 1976).

DISCUSSION

Throughout the 4 years that this equipment has been used (two separate complexes at two locations), no major problems have been encountered. Maintenance has been minimal, normally requiring fuse replacement and occasionally the replacement of Q1, the DTS-710 transistor, when voltage loads were inadvertently applied above their 900-V capacity.

Occasionally, when different gel and buffer formulations were used, we experienced trouble in maintaining the current to the maximums when four groups of six disk gels were run in the individual upper and lower bath arrangement. This problem resulted from the additive resistance of the gels surpassing the 900-V maximum. This condition was remedied, however, by run-

ning the gels at a lower amperage or by running less gels per station.

Extensive measurements made during electrophoresis runs showed that the constant-current regulator was accurate to ± 1 percent of the current setting throughout its range. These measurements were made by comparing an external, calibrated digital multimeter against the current and voltage measuring devices built into the constant-current regulators (options 1 and 2).

Extensive use of both constant-current regulators have shown that option 1, which utilizes digital meters, has an advantage over option 2, which utilizes analog meters. Option 1 more consistently and more precisely sets the desired initial running currents and monitors the stability of the current to each cell during a run.

The original constant-current regulator (option 2) was recently upgraded by replacing the analog meters with digital meters. Also, a sequencing controller was added to monitor the 12 positions by automatically switching through them every 20 seconds. The sequencing operation can be stopped at any of the 12 stations and started again with the push of a button (Ted Aldrich, 1976, personal communication).

The multifunctional electrophoresis system is primarily used as a stationary structure mounted in a 40-ft³ refrigerated cooler and has not been extensively used mounted on a cart. However, in the prototype model, the coolant system was tested in conjunction with the coolant-chamber electrophoresis cells.

Cold (5° C) water was circulated through the system at a rate of 50 ml per minute per cell. At maximum current (35 mA/cell), the cooling properties were excellent. The temperature of the water measured at the return manifold was usually at 6° to 8° C.

One difficulty that occurred sporadically was that the glass plates forming the acrylamide chamber of the cell would crack because of cold shock. This problem was alleviated by preconditioning the cells at around 15° C with circulating water or by filling the cell chambers with water near room temperature and the lower baths in which they sit with 5° C buffer prior to sample application. In the process of applying sample to the slots, the chamber water cooled sufficiently so that the circulator could be started at 5° C. These procedures did not result in gel shrinkage and separation from the glass cell.

One other difficulty was in the flow rate be-

tween the different tiers. The flow rate of the bottom tiers was always greater than that of the top tiers of stations. This difference was evident because the feed lines to the bottom stations were shorter than those in the top tiers. It was necessary to set the flow rates initially with adjustable pinch clamps. Once the flow rates were adjusted, no further change was necessary. However, small differences were still noticed when the valves were switched to the cell bypass position, but these differences were not great enough to cause major difficulties.

Performance and operation of the other equipment presented in this report has functioned well with no problems, except for an occasional leak that was easily repaired.

REFERENCES

- Akroyd, P.
1967. Acrylamide gel slab electrophoresis in a simple glass cell for improved resolution and comparison of serum proteins. *Anal. Biochem.* 19: 399-410.
- Allen, R. C., and Moore, D. J.
1966. A vertical flat-bed discontinuous electrophoresis system in polyacrylamide gel. *Anal. Biochem.* 16: 457-465.
- Besaw, L. C.; Dalby, P. L.; and Lillevik, H. A.
1972. A multicompartamental disc electrophoresis apparatus. *Anal. Biochem.* 50: 632-634.
- Brewer, G. J.
1970. An introduction to isozyme techniques, pp. 24-30. Academic Press, New York.
- Clarke, J. T.
1964. Simplified "disc" (polyacrylamide gel) electrophoresis. *Ann. N.Y. Acad. Sci.* 121: 428-436.
- Davis, B. J.
1964. Disc electrophoresis. II. Method and application to human serum proteins. *Ann. N.Y. Acad. Sci.* 121: 404-427.
- McDonald, I. C.
1976. Ecological genetics and the sampling of insect populations for laboratory colonization. *Environ. Entomol.* 5: 815-820.
- McDonald, I. C., and Johnson, O. A.
1976. Isozyme variability studies of translocation homozygotes in the house fly, *Musca domestica* L. *Proc. Int. Conf. Entomol.*, Aug. 19-27, 1976, Washington, D.C., pp. 140-145.
- McDonald, I. C.; Terranova, A. C.; Johnson, G. A.; and Leopold, R. A.
1975. Polymorphisms and inheritance patterns of tetrazolium oxidase and octanol dehydrogenase in the house fly. *J. Hered.* 66: 218-220.
- Maurer, H. R.
1974. Requirements of reproducible and standardizable polyacrylamide gel electrophoresis. *In* R. C. Allen and H. R. Maurer (eds.), *Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel*, pp. 23-37. Walter de Gruyter, New York.
- Maurer, H. R., and Dati, F. A.
1972. Polyacrylamide gel electrophoresis on micro slabs. *Anal. Biochem.* 46: 19-32.
- Morris, C. J. O. R., and Morris, P.
1974. Physico-chemical measurements in polyacrylamide gels. *In* R. C. Allen and H. R. Maurer (eds.), *Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel*, pp. 9-15. Walter de Gruyter, New York.
- Raymond, S.
1962. A convenient apparatus for vertical gel electrophoresis. *Clin. Chem. (Winston-Salem, N.C.)* 8: 455-470.
- Rodbard, D., and Chrambach, A.
1974. Quantitative polyacrylamide gel electrophoresis: Mathematical and statistical analysis of data. *In* R. C. Allen and H. R. Maurer (eds.), *Electrophoresis and Isoelectric Focusing in Polyacrylamide Gel*, pp. 28-62. Walter de Gruyter, New York.
- Woodworth, R. C., and Clark, L. G.
1967. An improved vertical polyacrylamide gel electrophoresis apparatus: Application to typing and subtyping of haptoglobins. *Anal. Biochem.* 18: 295-304.

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